NOVEL TEMPLATE MOTIFS FROM CODED AMINO ACIDS

A Thesis Submitted
in Partial Fulfilment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

by
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to the

DEPARTMENT OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY, KANPUR

June, 1994

STATEMENT

I hereby declare that the matter embodied in this thesis is the result of investigatio carried out by me in the Department of Chemistry, Indian Institute of Technolog Kanpur, India, under the supervision of Professor S. Ranganathan.

In keeping with the general practice of reporting scientific observations, due acknow edgements have been made wherever the work embodied is based on the findings of othe investigators.

IIT Kanpur
June, 1994

(N.TAMILARASU)

CERTIFICATE

Certified that the work contained in this thesis, entitled, "NOVEL TEMPLAT
MOTIFS FROM CODED AMINO ACIDS" has been carried out by Mr. N.Tamilaras
under my supervision and the same has not been submitted elsewhere for a degree.

IIT Kanpur
June, 1994

(S. Ranganathan)
Thesis Supervisor

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CERTIFICATE OF COURSE WORK

This is to certify that Mr. N. Tamilarasu (Roll number 8910771) has satisfactorily completed all the courses required for the Ph.D. degree programme. These courses include:

CHM 605	Principles of Organic Chemistry
CHM 625	Principles of Physical Chemistry
CHM 645	Principles of Inorganic Chemistry
CHM 624	Modern Physical Methods in Chemistry
CHM 602	Advanced Organic Chemistry
CHM 681	Basic Biological Chemistry
CHM 800	General Seminar
CHM 801	Special Seminar
CHM 900	Post Graduate Research

Mr. N. Tamilarasu has successfully completed his Ph.D. qualifying examination in September, 1990, he also successfully presented his open seminar of the work embodied in this thesis.

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Finally, I express my heartfelt gratitude to my parents and other members of my family for their constant encouragement and support.

N. TAMILARASU

ABSTRACT

The thesis describes endeavours directed at the construction of templates, designed for metal ion uptake, relevant to the biology domain, from coded amino acids.

None of the coded amino acids has independent copper uptake potential. In the multitudes of copper enzymes, the metal binding is accomplished by a stretch of residues as autonomous regions, wherein the contact amino acid side chains, such as, histidine, methionine, tyrosine, aspartic acid and cysteine are correctly positioned. Thus, from vantage of a minimalistic approach, the transformation of a coded amino acid to one having independent copper uptake potential, which, at the same time, could be incorporated readily in normal peptide synthesis, was considered attractive. This objective has been accomplished. The coded amino acid tyrosine has been modified into one having potential for direct uptake of not only Cu(II), but also Co(II) and Ni(II) and which, at the same time, can be readily incorporated into peptides.

L-Tyrosine was transformed to 3-acetyl tyrosine (Tyr-3-Ac) and its incorporation in regular peptide synthesis established. Although appeared promising, the o-hydroxy acetophenone unit in Tyr-3-Ac did not form salts with metal ions. This was accomplished on transformation of the 3-Ac carbonyl oxygen to a nitrogen equivalent.

Tyr-3-Ac side chains in peptides readily form oximes, which, on treatment with metal ions, form templates with great ease.

Of particular relevance is the efficient condensation of acetyl acetone - ethylenediamine mono Schiff base (AEII) with Tyr-3-Ac side chains in peptides. The resulting compounds readily form metal templates. Thus a single, strategically positioned, Tyr-3-Ac residue, on condensation with AEII, would provide site for metal ion uptake.

A novel secondary structural motif in proteins would be the bridging of proximate Tyr-3-Ac residues leading to pro-templates that have metal ion uptake potential. The feasibility of this has been demonstrated by linking of pairs of Tyr-3-Ac residues, in a peptide environment, with ethylenediamine. The resulting motifs readily form metal templates.

The ready acylation of Tyr, which led to useful chemistry, if applied to 3,4-dihydroxyphenylalanine (DOPA), could give rise to templates similar to that described above, and, in addition, to novel bi-metallic systems by attachment of ionophore arm to the extra hydroxyl functions. In the event, all attempts to acylate DOPA failed. An alternate strategy envisaged the attachment of a pro-template ligand to one of the hydroxyl groups of DOPA. Of the many substrates tried, only dimethyl bromo malonate gave the 3-O-alkylated product in poor yields. The attempted, one step transformation of DOPA to an ionophore by bis-MEM chloride alkylation afforded only the mono 4-alkylated product. An alternate allylation strategy afforded all the isomers, the yield of the bis-O-allyl compound being a modest 9 percent.

The efficient linking of the Tyr-3-Ac units with ethylenediamine to templates that have excellent metal uptake profile, generated the notion that were such a linking be brought about with cystine-di-OMe [$H_2NCH(COOR)CH_2SSCH_2CH(COOR)NH_2$], the resulting bis-Schiff base on S-S reduction on complexation can lead to bi-metallic clusters of the type, $Zn_2[SR]_2OR_2[=NR]_2$, wherein, both the S atoms are coordinated to both the metal centres, a profile in common with Gal 4 recognition motif. The Gal 4 protein (881 residues), turn on the genes that carry the information for the metabolism of galactose and mellibiose. The compact metal binding domain, which directly recognises a DNA triplet sequence, consists of 30 residues that generate a bi-metallic Zn cluster, involving six cysteines, of which two are shared.

Tyr-3-Ac failed to react with either cystine-di-OMe, or S-acetamidomethyl cysteine-

OMe. However, salicylaldehyde readily afforded the expected bis-Schiff base with cystine-di-OMe. This on reduction with PDT followed by complexation with zinc afforded a metal complex, whose nature, although in agreement with the expected zinc cluster motif, remains to be fully established.

The replacement of nitrogen and oxygen ligands in the above cluster with sulfur would lead to $[Zn_2SR_6]^{2-}$, motif closer to that in Gal 4. This gave rise to the realization that such an ensemble can be formed if the aromatic substrates here are replaced by cystine and pair of these linked with cystine. The resulting motif on reduction and metal complexation can lead to the desired goal.

Tandem condensation of three cystines, was readily accomplished to afford novel peptide, wherein, within the twenty two atom frame work are inscribed three di-sulfide and two peptide bonds. Detailed spectral studies have shown that it is associated in non polar solvents like chloroform and intramolecularly hydrogen bonded as monomeric structures in DMSO. The incorporation of zinc here via PDT reduction and complexation did not succeed, probably because of unfavourable alignment of the three di-sulfide bonds.

CONTENTS

$\star\star\star$

ABBREVIATIONS

Ac	acetyl
AEH	acetylacetone - ethylenediamine mono-schiff base
Ala	alanine
ATP	adinosine triphosphate
Boc	t-butyloxycarbonyl
Bz	benzoyl
CD	circular dichroism
Cys	cysteine
DCC	dicyclohexylcarbodiimide
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DOPA	3,4-dihydroxyphenylalanine
DPPH	dipicrylphosphoryl azide
EDA	ethylenediamine
EDTA	ethylenediaminetetraacetic acid
EEDQ	ethyl-1,2-dihydroxy-2-ethoxy-1-quinoline carboxylate
EPR/epr	electron paramagnetic resonance
Et	ethyl
FAB	fast atom bombardment
HOBt	1-hydroxybenzotriazole
IR/ir	infra-red
LNT	liquid nitrogen temperature

Me	methyl
mp	melting point
ms	mass spectrum
M.W.	molecular weight
NMR/nmr	nuclear magnetic resonance
NOE	nuclear overhauser effect
NOESY	nuclear overhauser effect spectroscopy
PDT	1,3-propanedithiol
Pep	peptide
Ph	phenyl
ppb	parts per billion
PPM/ppm	parts per million
Py	pyridine
tRNA	transfer ribonucleic acid
rt	room temperature
Ser	serine
tlc	thin layer chromatography
Ts	p-toluenesulphonyl
Tyr	tyrosine
UV/uv	ultra-violet
VIS/vis	visible
VT	variable temperature
Z	benzyloxycarbonyl

A. INTRODUCTION

Any form of life in this planet would not have evolved but for the availability for incorporation into the carbon manifold a variety of metal ions. From the control of the neural responses via modulation of passage of sodium and potassium ions, to the transport of oxygen via iron, the harnessing of energy from the Sun using a variety of metal ions such as magnesium, manganese, copper and iron, to the potential cascade associated with the transformation of chemical energy of bonds to ATP involving copper and iron, to that of the assimilation of CO_2 and protein-DNA recognition by zinc and in the manifestation of several critical biological processes using a range of metal ions such as cobalt, aluminium, calcium, iron, copper, they form the integral part of life system, which is an epitome' of symbiosis of the information system with the functional system, with versatility to use energy in many of its manifestations such as chemical, electrical, mechanical, osmatic and hydrostatic. In this exotic scenario copper ions play a stellar role and consequently the structural environments that are associated has been the focus of many investigations.

The genesis of the present work pertains to crafting a link between the coded amino acids and this metal ion, an option not available to Nature. The work outlined in the thesis has successfully demonstrated that the coded amino acid tyrosine (Tyr) can be modified into one having potential for uptake of metal ions such as Cu, Ni, Co and which could be readily incorporated into peptides. Logical leads arising from this endeavour, namely, the generation of metal clusters in a protein manifold and the possible transformation of L-3,4-dihydroxyphenylalanine (L-DOPA) to systems that can harbor metal ions have also been explored. These have been presented in SECTION.C. As an appropriate background to the present work the chemistry of tyrosine and 3,4-dihydroxyphenylalanine (DOPA) has been briefly reviewed and presented in SECTION.B.

B. BACKGROUND

CHEMISTRY OF TYROSINE :

L-Tyrosine is an important component of many biologically active peptides[1] and is a precursor of catecholamines in living organism.[2] At the same time, it is an important constituent of polypeptide metalloenzymes, since, a large proportion of this class of enzymes harbour tyrosine residues at the metal binding sites.[3]

The biosynthesis[4] of L-adrenaline from L-tyrosine proceeds via hydroxylation to 3,4-dihydroxyphenylalanine (DOPA), decarboxylation to dopamine, β-hydroxylation to noradrenaline and N-methylation (CHART B.1). The complex organic chemistry associated with these changes are brought about by a unique enzyme ensemble. DOPA on its own right occupies a pre-eminent position in cellular metabolism. The Tyr $\rightarrow$ DOPA change can be accomplished either via the 3-nitro derivative (CHART B.2)[5] or via 3-Ac-Tyr (CHART B.3).[6] As shown in CHART B.3, the key 3-Ac-Tyr can be prepared either directly from tyrosine by Fridel-Crafts reaction or from the acetate by Fries rearrangement.[6] The chemical simulation of the Tyr $\rightarrow$ DOPA change has been claimed[7] on treatment with EDTA/$FeSO_4$ at 0° and pH 6, on N-protected Tyr (CHART B.4). CHART B.2 and CHART B.3 outlined the 3-location of tyrosine. Adaptation of this strategy to prepare 4-O-protected DOPA has been found to be not so easy. This O-benzylation of 3-nitro-tyrosine followed by reduction and diazotization afforded the rather interesting 4-O-benzyl-3-diazonium tyrosine. The latter could not be transformed to 4-O-benzyl-DOPA (CHART B.5).[8] However 3-acetyl-tyrosine could be protected, reduced and subjected to rearrangement in presence of H_2O_2-TsOH to afford 4-protected DOPA (CHART B.6).[9]

The nitration of tyrosine as described above, requires drastic reaction conditions not at all suitable in a protein environment. In this context the finding that tyrosine is

CHART B.1

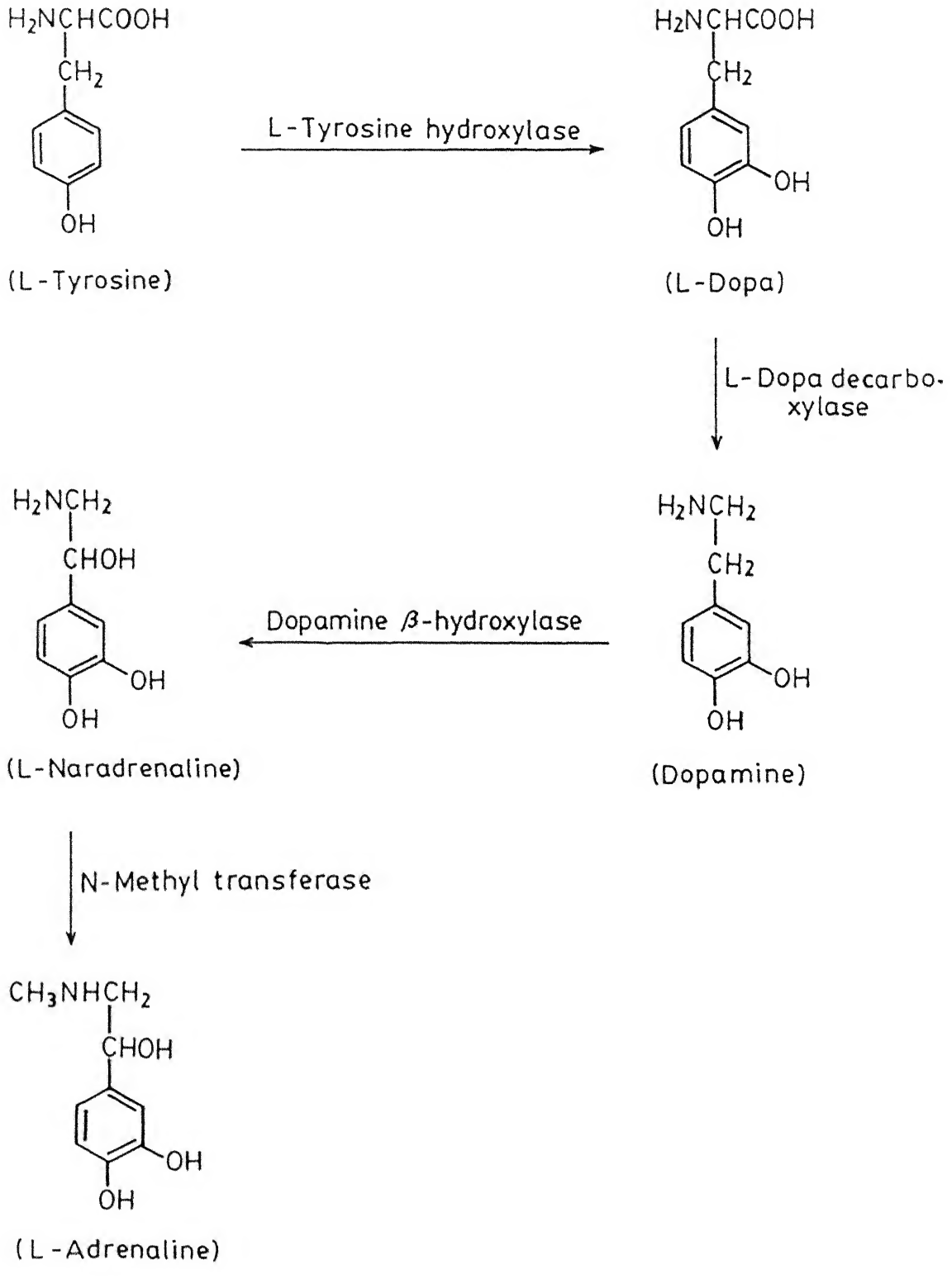

H2NCHCOOH
CH2
OH
(L-Tyrosine)
L-Tyrosine hydroxylase
H2NCHCOOH
CH2
OH
OH
(L-Dopa)
L-Dopa decarbo-
xylase
H2NCH2
CH2
OH
OH
(Dopamine)
Dopamine β-hydroxylase
H2NCH2
CHOH
OH
OH
(L-Naradrenaline)
N-Methyl transferase
CH3NHCH2
CHOH
OH
OH
(L-Adrenaline)

CHART B.2

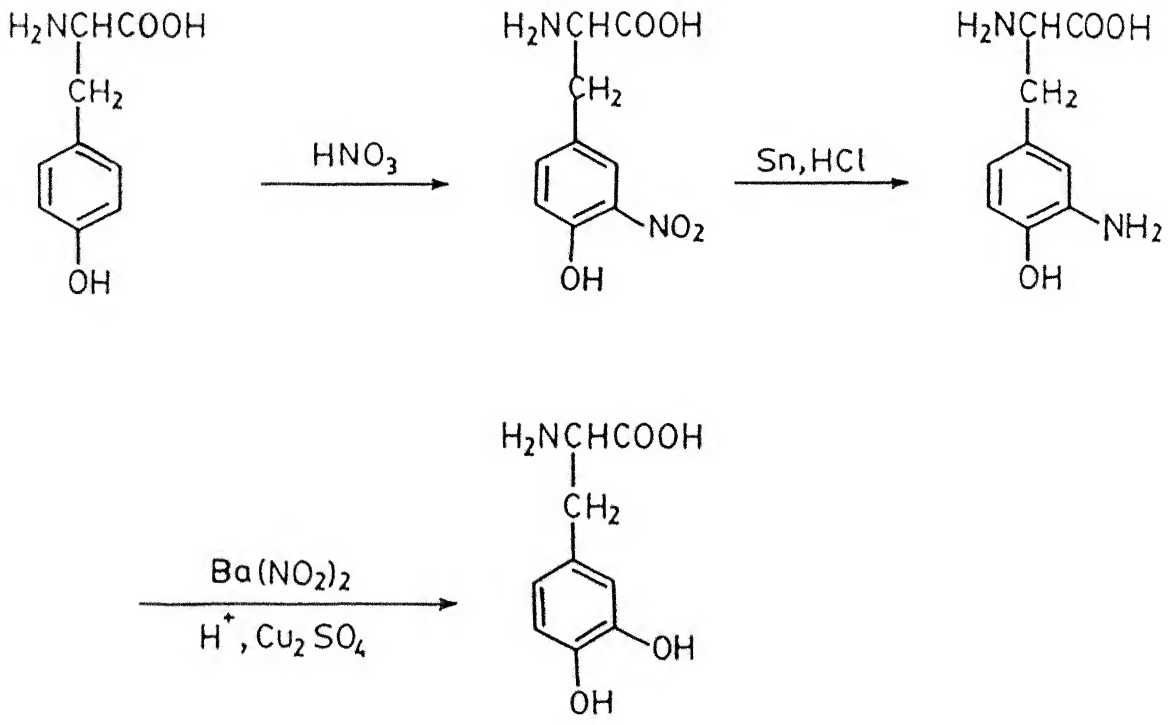


CHART B.3

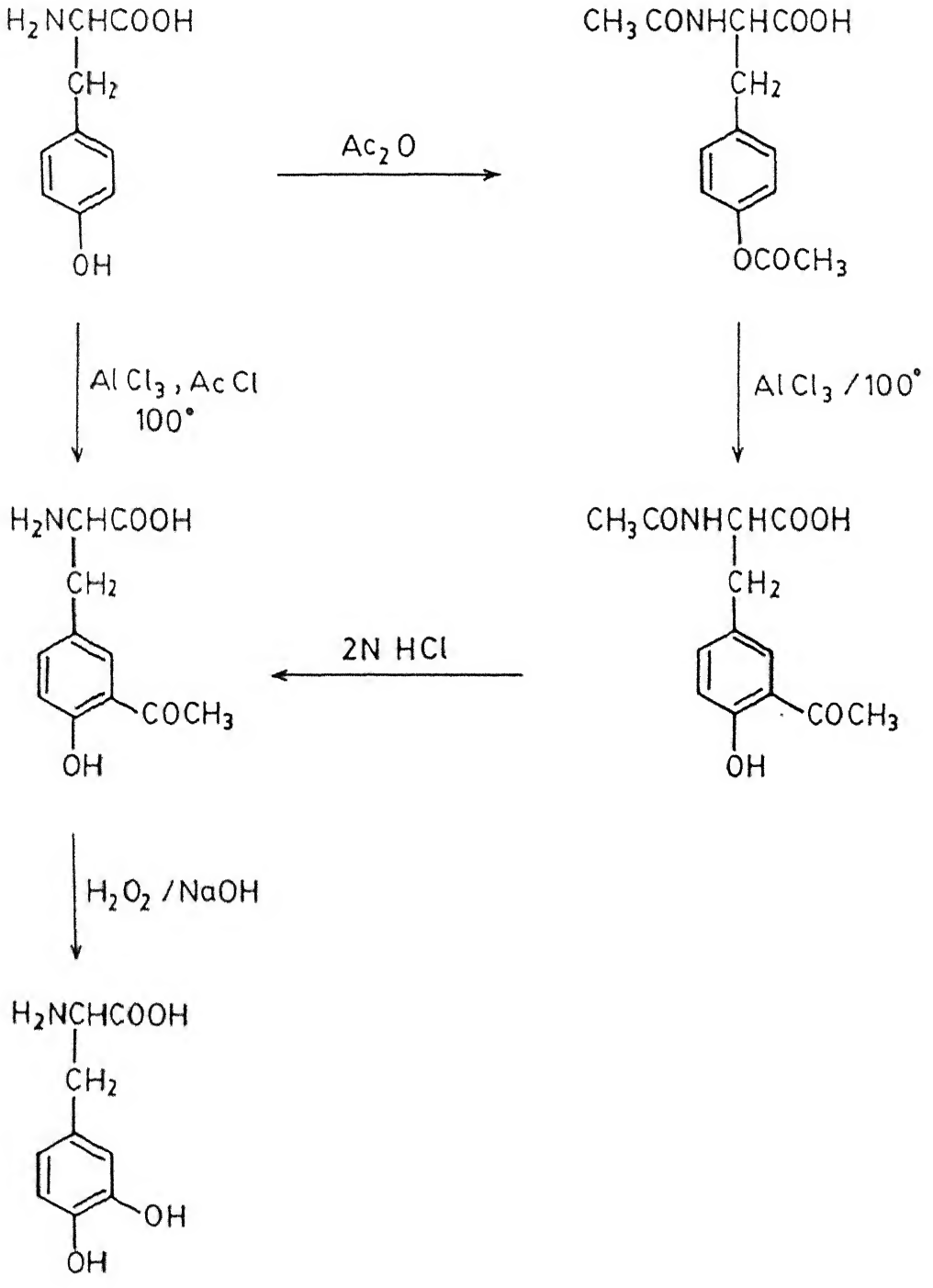
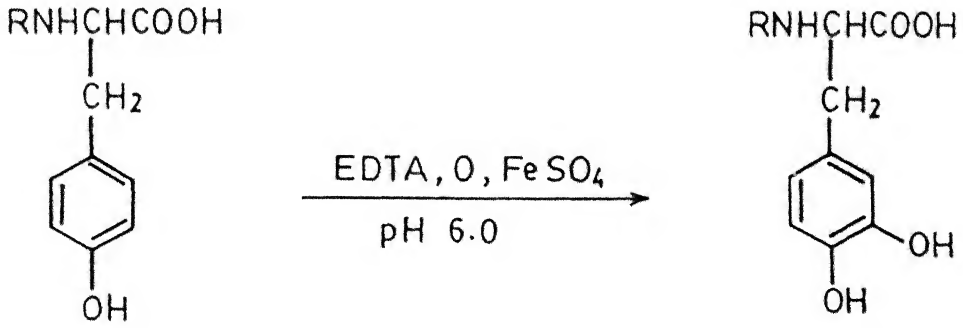

H2NCHCOOH
CH2
OH
Ac2O
CH3CONHCHCOOH
CH2
OCOCH3
AlCl3, AcCl
100°
AlCl3 / 100°
H2NCHCOOH
CH2
COCH3
OH
2N HCl
CH3CONHCHCOOH
CH2
COCH3
OH
H2O2 / NaOH
H2NCHCOOH
CH2
OH
OH

CHART B.4



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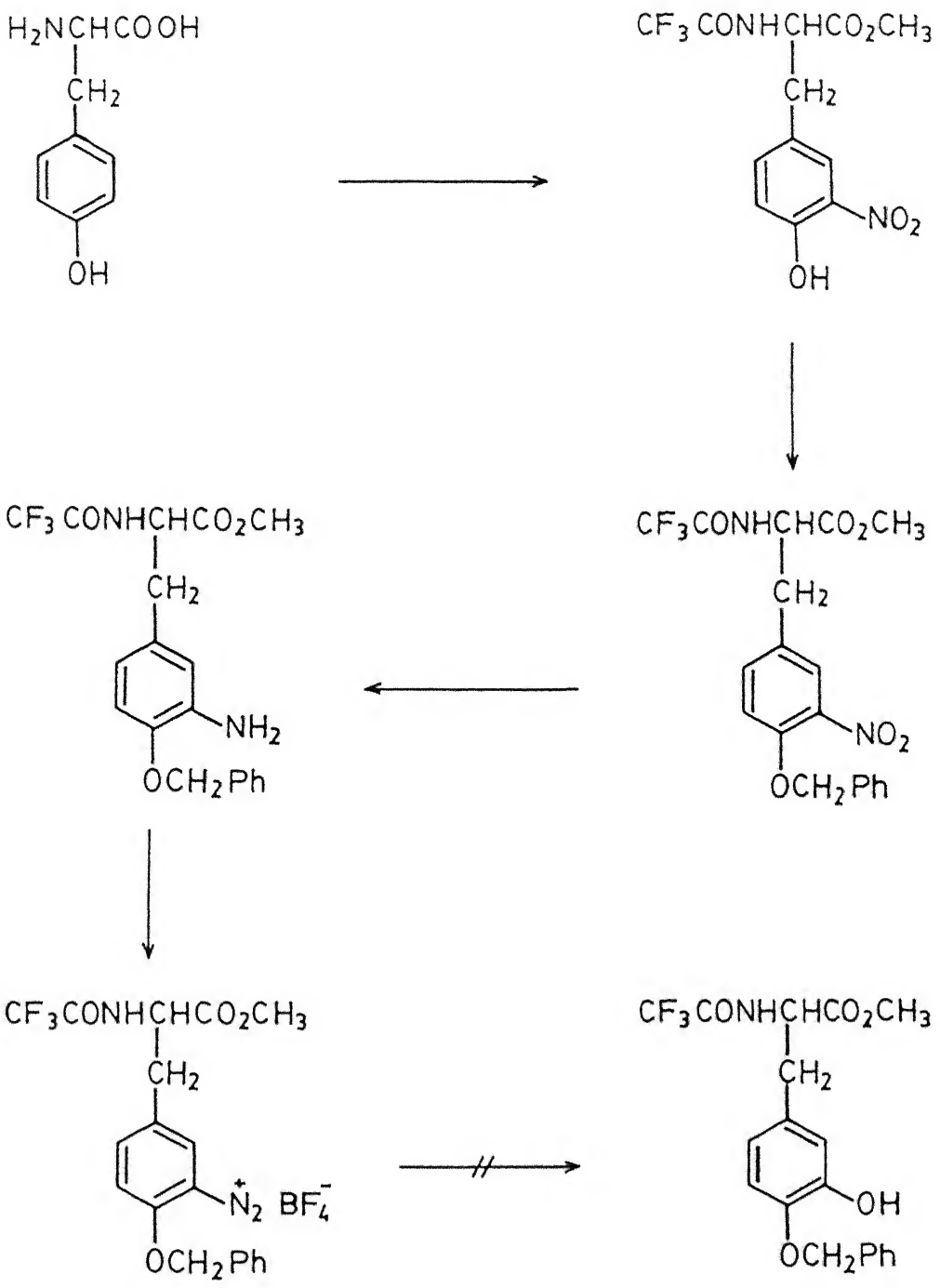
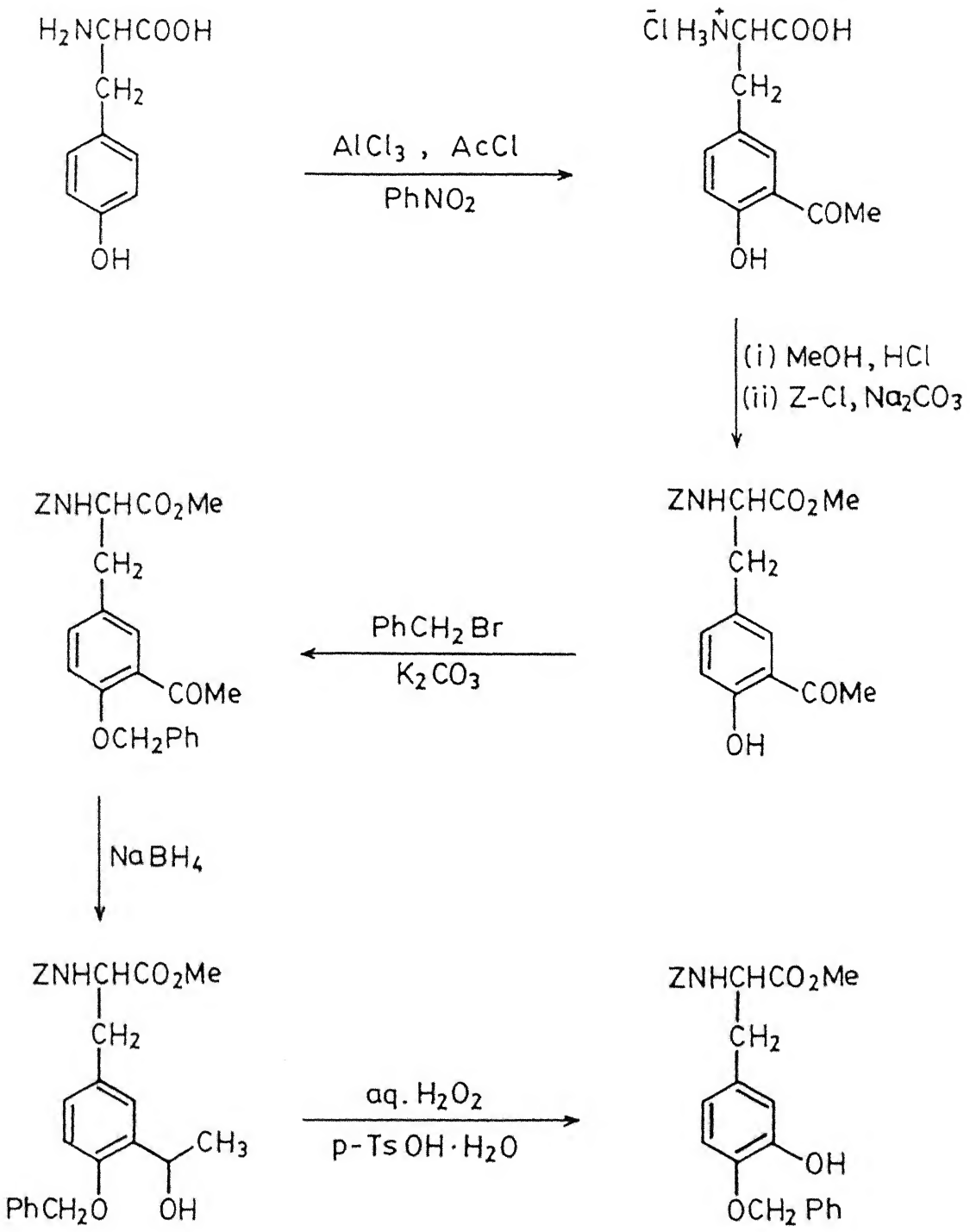

H2NCHCOOH
CH2
OH
CF3CONHCHCO2CH3
CH2
NO2
OH
CF3CONHCHCO2CH3
CH2
NO2
OCH2Ph
CF3CONHCHCO2CH3
CH2
NH2
OCH2Ph
CF3CONHCHCO2CH3
CH2
N2+ BF4−
OCH2Ph
CF3CONHCHCO2CH3
CH2
OH
OCH2Ph

CHART B.6


H2NCHCOOH
CH2
OH
AlCl3 , AcCl
PhNO2
Cl H3NCHCOOH
CH2
COMe
OH
(i) MeOH, HCl
(ii) Z-Cl, Na2CO3
ZNHCHCO2Me
CH2
COMe
OH
PhCH2Br
K2CO3
ZNHCHCO2Me
CH2
COMe
OCH2Ph
NaBH4
ZNHCHCO2Me
CH2
CH3
PhCH2O
OH
aq. H2O2
p-TsOH·H2O
ZNHCHCO2Me
CH2
OH
OCH2Ph

specifically transformed to 3-nitro-tyrosine at room temperature at pH 8 is of practical significance (CHART B.7).[10] Coded amino acids other than cysteine are not affected by the reagent and therefore this reagent holds promise in the introduction of the nitro group in tyrosine in proteins and peptides. Nitration extends the number of available procedures for the chemical modification of tyrosyl residues, providing greater flexibility for the study of their role in the biological function of proteins. Further, reduction of the nitro to an amino group may lead to yet additional derivatives. Nitration should also facilitate identification of "tyrosyl" enzymes, those in which tyrosyl groups are involved in enzymatic activity.

Indeed [2-(3-nitro-L-tyrosine)]oxytocin has been prepared from oxytocin on treatment with $C(NO_2)_4$ (CHART B.8)[11].

N-Protected 3-nitro-tyrosine would have a free COOH as well as a phenolic OH. Self condensation of these two can be brought about with DCC to polymeric, granular active polyesters. These are highly versatile in the transfer of the 3-nitro-tyrosine unit to the N-terminal positions of peptides (CHART B.9).[12]

Tyr-3-COOH occurs in the seeds of *roseda orderata L.* Synthesis has been accomplished from 3-nitro-tyrosine, via reduction, diazotization, CuCN treatment and hydrolysis (CHART B.10).[13] An alternate procedure obviating the low yield Sandmayer reaction involves 3-formylation of fully protected tyrosine (CHART B.11).[14]

Electrophilic substitution of tyrosine with $HOOC\text{-}CH(^{+}NH_3)\text{-}CH_2S^{+}$ has been accomplished in low yields in presence of boiling 47% HBr (CHART B.12).[15] Such Tyr-Cys composites are potential cytotoxic agents and their preparation in good yields would be of interest.

3-O-Alkyl-tyrosines can be directly prepared from tyrosine in DMSO and alkyl halides in presence of dilute NaOH. Interestingly in presence of concentrated alkali O-alkyl esters

CHART B.7

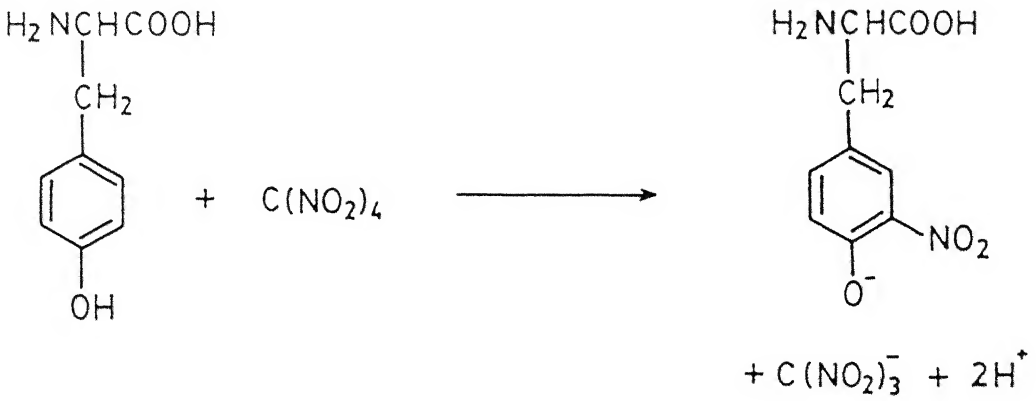


CHART B.8

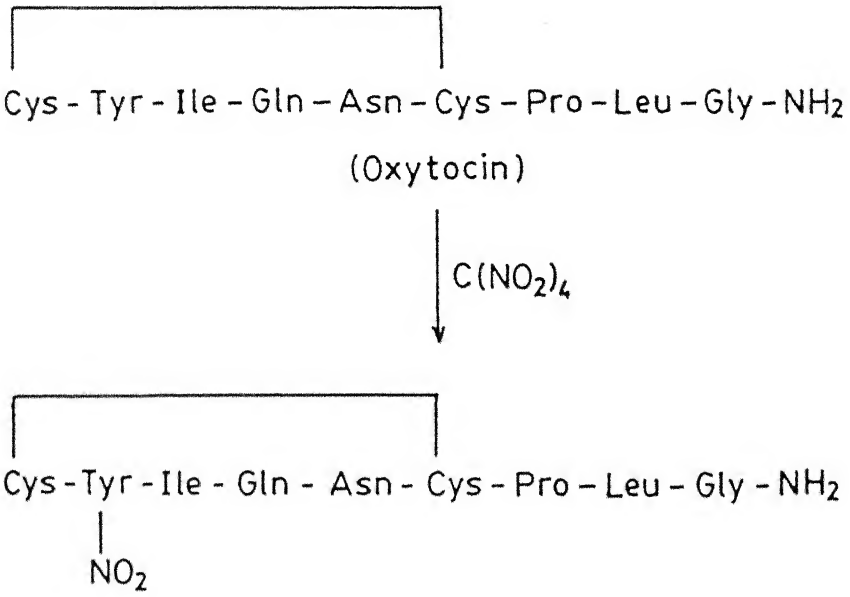


CHART B.9

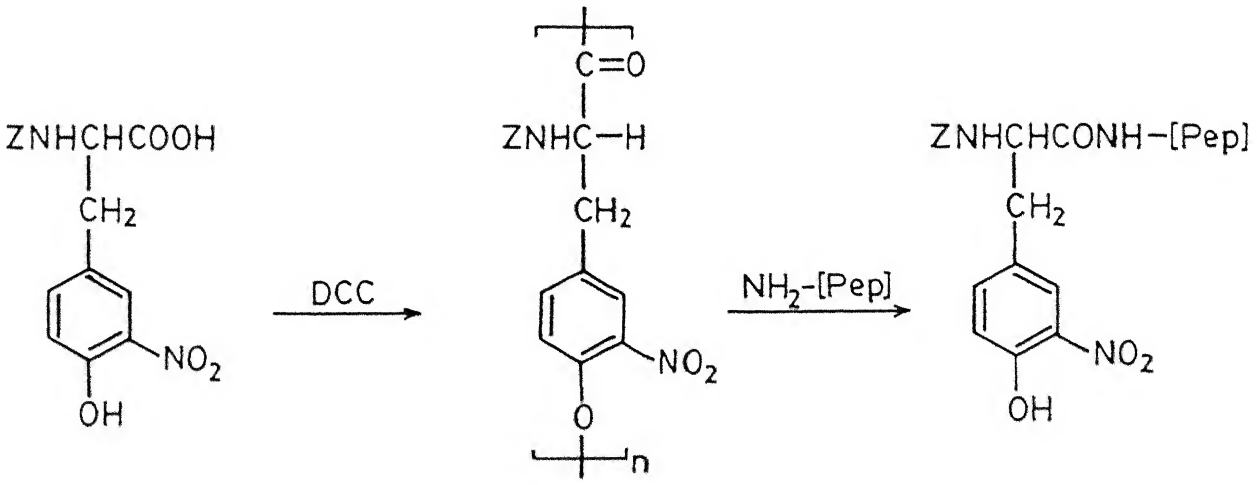

ZNHCHCOOH
CH2
NO2
OH
DCC
C=O
ZNHC—H
CH2
NO2
O
n
NH2-[Pep]
ZNHCHCONH—[Pep]
CH2
NO2
OH

CHART B.10

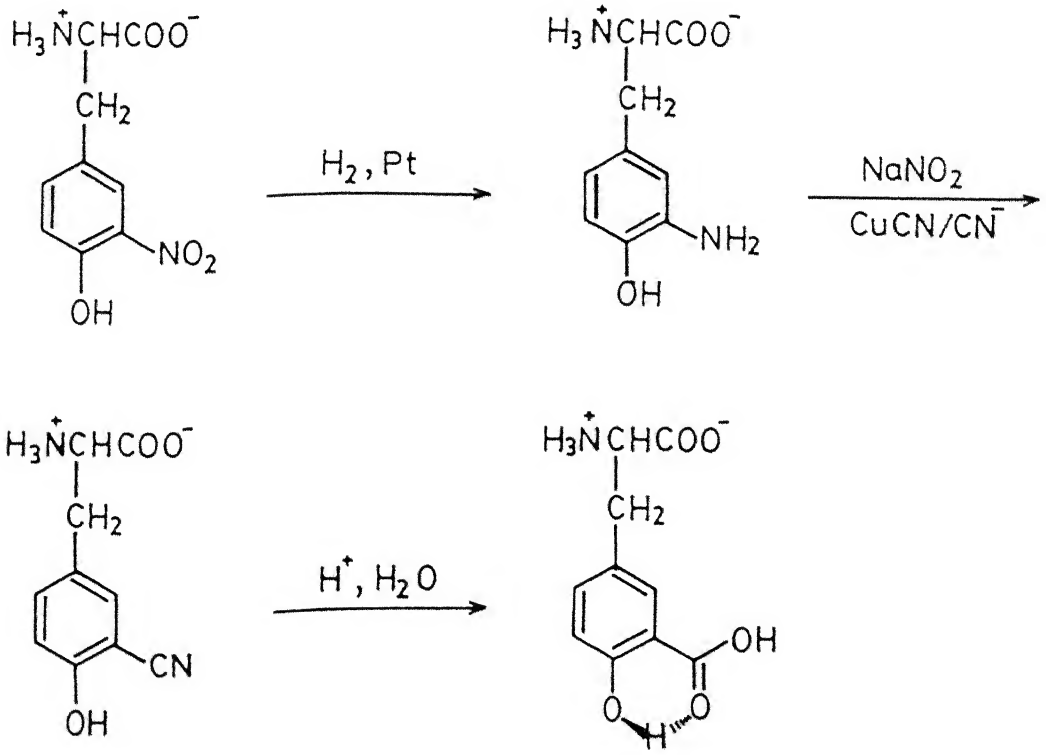


CHART B.11

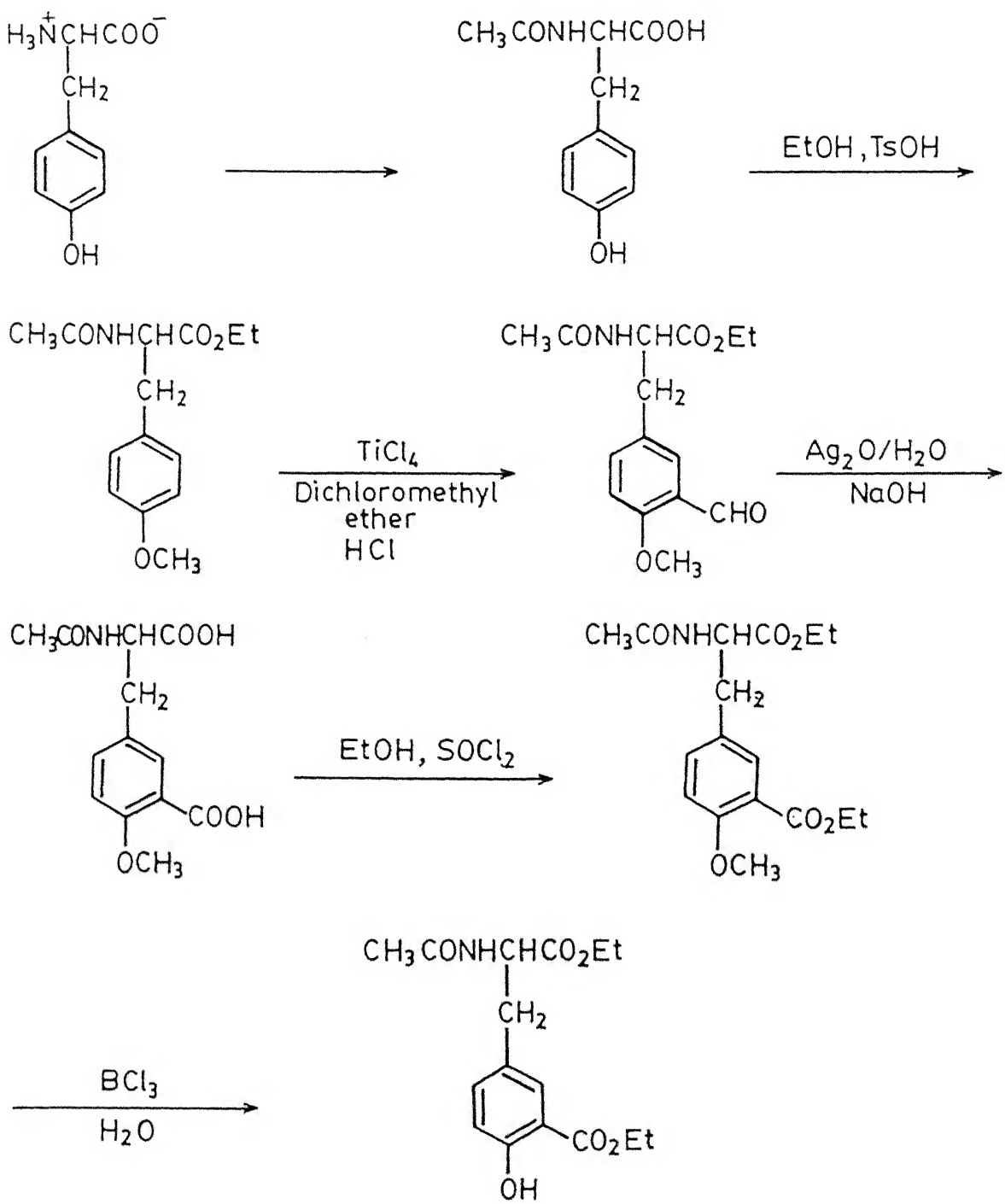
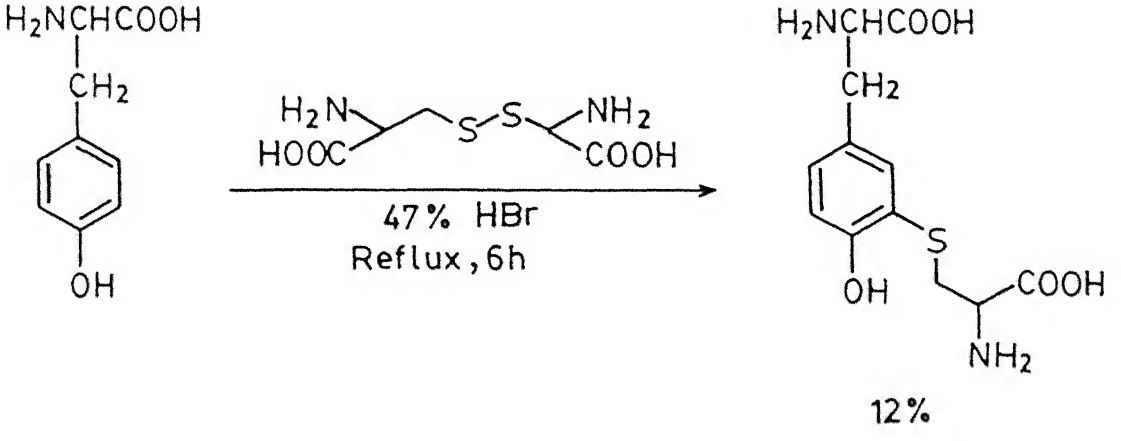

H3N+CHCOO−
CH2
OH
CH3CONHCHCOOH
CH2
OH
EtOH, TsOH
CH3CONHCHCO2Et
CH2
OCH3
TiCl4
Dichloromethyl ether
HCl
CH3CONHCHCO2Et
CH2
CHO
OCH3
Ag2O/H2O
NaOH
CH3CONHCHCOOH
CH2
COOH
OCH3
EtOH, SOCl2
CH3CONHCHCO2Et
CH2
CO2Et
OCH3
BCl3
H2O
CH3CONHCHCO2Et
CH2
CO2Et
OH

CHART B.12


H2NCHCOOH
CH2
OH
H2N
HOOC
S
S
NH2
COOH
47% HBr
Reflux, 6h
H2NCHCOOH
CH2
OH
S
COOH
NH2

12%

are formed (CHART B.13).[16]

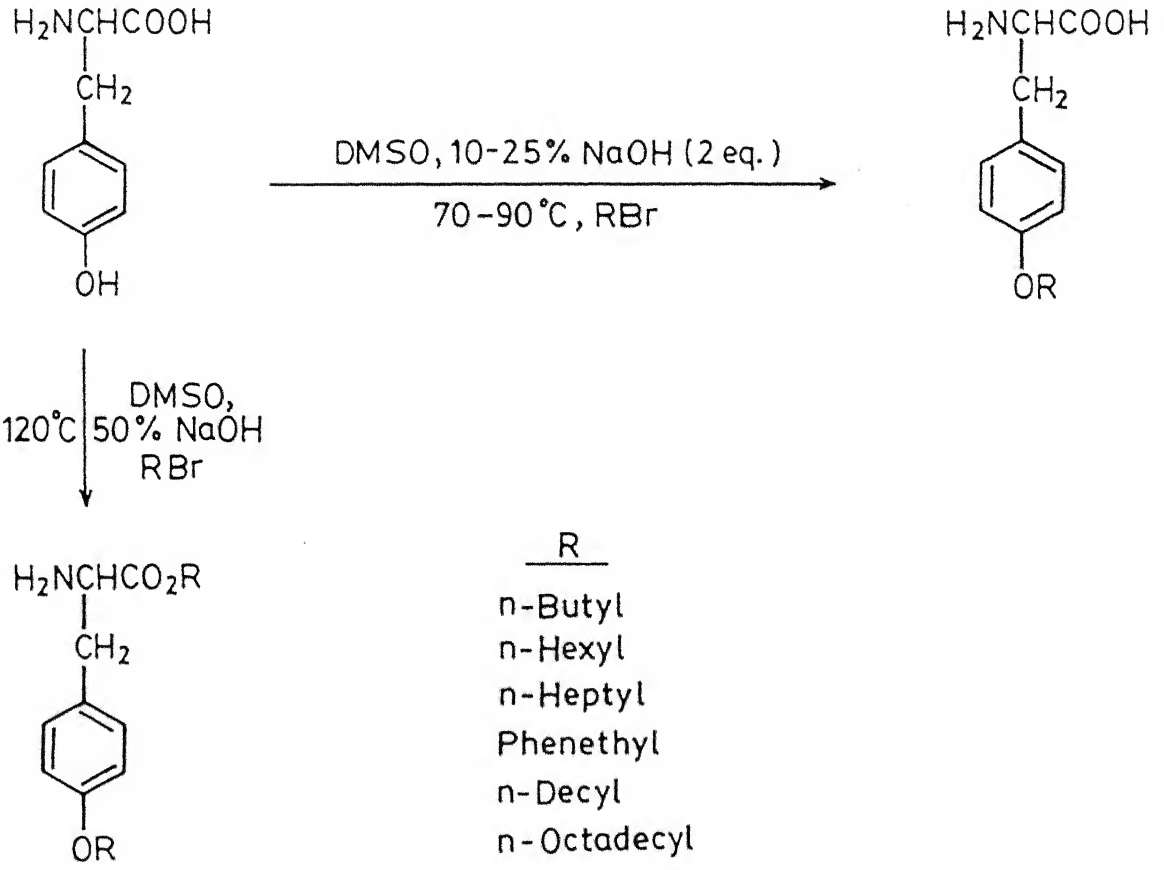
O-Epoxyalkyl-L-tyrosines prepared via O-alkylation of protected tyrosine followed by epoxidation, are specific and irreversible inhibitors of the proteases, subtilisin and α-chymotrypsin (CHART B.14).[1]

The phosphorylation of proteins is recognised as an important regulatory mechanism for numerous physiological processes while less abundant than serine- or threonine-phosphorylation, tyrosine phosphorylation is of great significance as it has been linked with the malignant transformation of cells by some RNA tumour viruses.

O-Phosphorylation of tyrosine has been achieved in a clean manner on treatment with pyrophosphoric acid at 80°C for 24 hours (CHART B.15).[17] A number of studies have been focused on the co-ordination behaviour of tyrosine and tyrosine analogs. Through a combination of the usual potentiometric, calorimetric, uv-visible spectrophotometry, optical rotatory dispersion and nmr techniques it was possible to reveal the protonation and some metal-ion derivatives at both macroscopic and molecular levels. 3-Amino tyrosine presents four sites for metal co-ordination. A pH-metric and spectroscopic study has been made of the proton and Cu(II) complexes of 3-amino-L-tyrosine and it has been established that various monomeric complexes involving aminocarboxylate and aminophenolate- type co-ordination, and dimeric complexes involving simultaneous metal-ion coordination at both bonding sites, are formed (CHART B.16).[18]

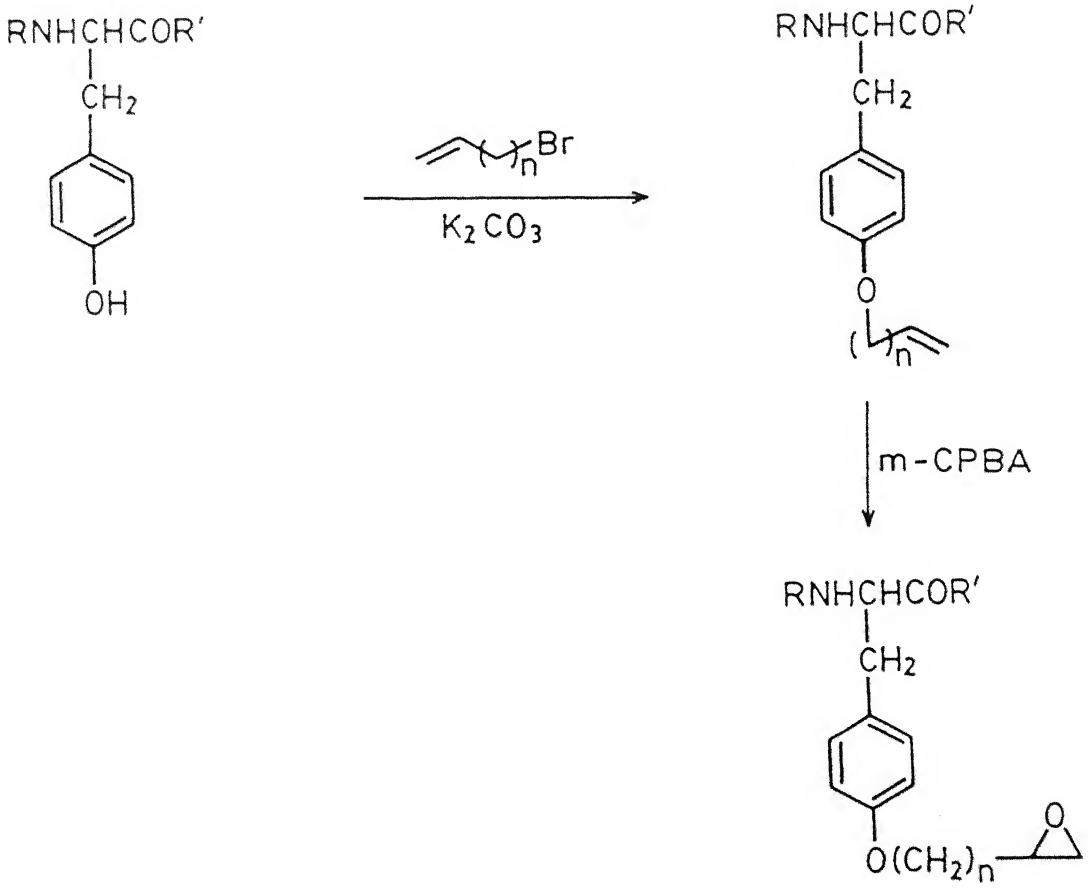
The involvement of metal ions in the biological reactions of tyrosine[19] has resulted in the synthesis and structural studies of complexes of L-tyrosine hydrazide with Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) (CHART B.17)[20].

CHART B.13



R
n-Butyl
n-Hexyl
n-Heptyl
Phenethyl
n-Decyl
n-Octadecyl

CHART B.14



R	R'
CH_3CO	OEt
C_6H_5CO	OEt
C_6H_5CO	-NH-p-$C_6H_4NO_2$

CHART B.15

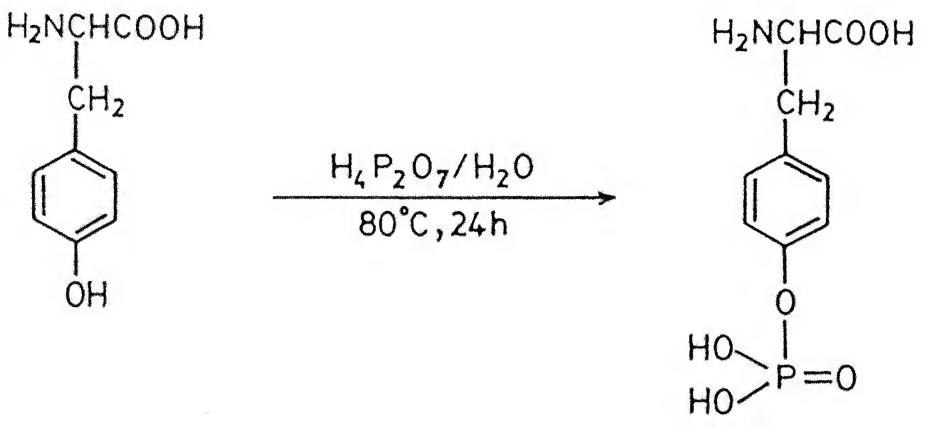
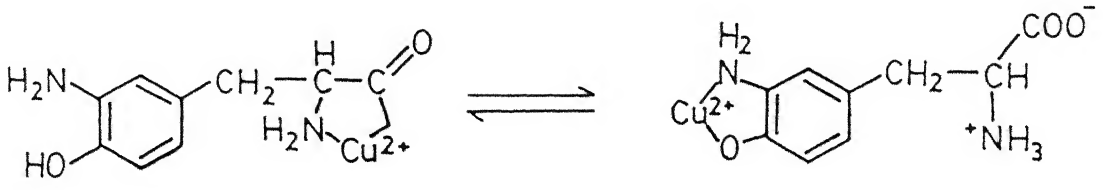
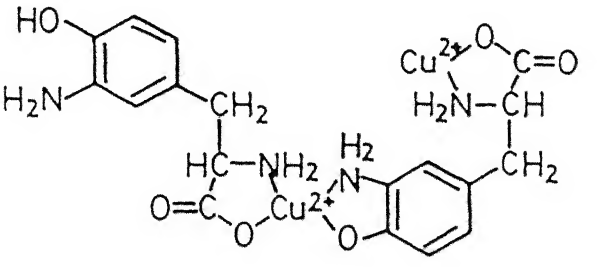


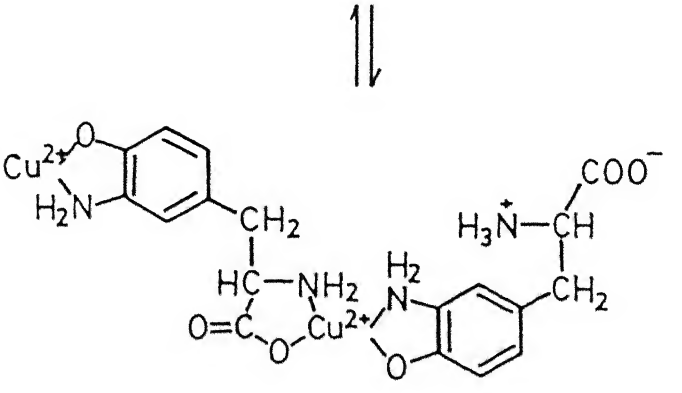
CHART B.16

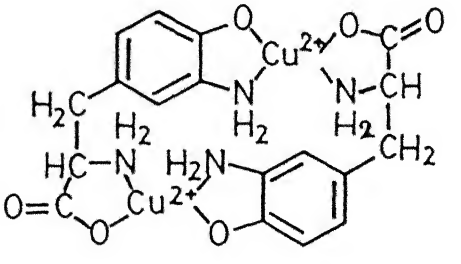


$[Cu(HA)]^+$



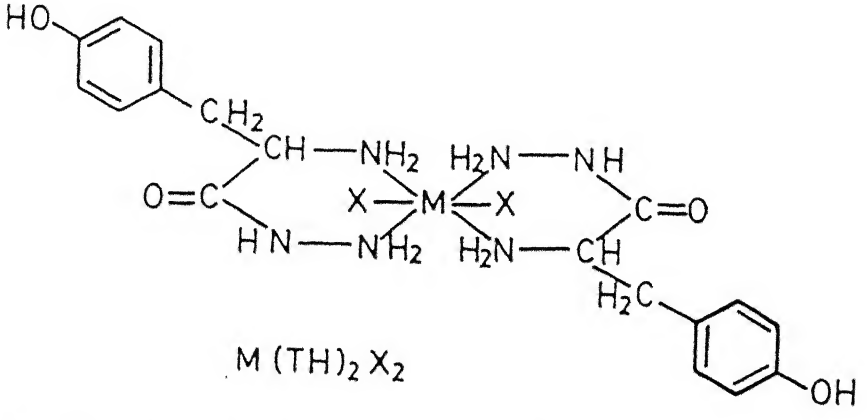
$[Cu_2A_2H]^+$





$[CuA_2]$

CHART B.17



$M(TH)_2X_2$

[X=Cl or OH; M=Mn(II), Co(II), Ni(II), Cu(II), Zn(II)]

CHEMISTRY OF 3,4-DIHYDROXYPHENYLALANINE (L-DOPA) :

3,4-Dihydroxyphenylalanine (L-DOPA) is the substrate of several enzyme systems such as dopa oxidase and dopa decarboxylase (CHART B.1) and is involved in the formation of several metabolic products such as melanins[21] and to 3,4-dihydroxyphenethylamine and norepinephrine.[22]

L-DOPA is still generally accepted as the first drug choice in the management of Parkinsonism. Long term therapy with L-DOPA is, however associated with a number of therapeutic problems.[23] The most serious limitations of L-DOPA can be summarized as follows: poor bioavailability, wide range of interpatient variations of plasma levels, unpredictable therapeutic response, and various side effects. The main factors responsible for these problems are the physical-chemical properties of the drug substance: low water solubility resulting incomplete dissolution at and prior to the absorption site, low lipid solubility resulting in unfavourable partition, and the high susceptibility of the drug molecule to chemical and enzymatic degradation.[24]

L-DOPA is usually administered orally, and in fact the drug is extensively metabolized in the gastrointestinal tract and/or during its first passage through the liver, so that relatively little arrives in the blood as intact L-DOPA. This metabolism of L-DOPA is unfavourable to its therapeutic intent.[25] L-DOPA is rapidly and continuously metabolized in blood, since only 5-8% of it is protein bound, making it very susceptible to metabolic processes.[26] An ideal prodrug of L-DOPA should be soluble in water and lipids, completely adsorbed from the gastrointestinal tract without any chemical degradation or metabolism, and thus deliver L-DOPA intact in the blood stream, at a reproducible therapeutic level. Consistent endeavours are being made in this direction, although an optimum based drug has thus far eluded discovery.

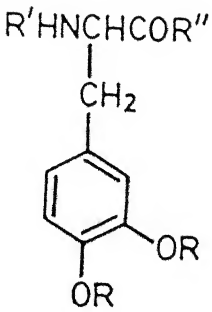
The three functional groups in DOPA, namely carboxy function, amino group and the catechol system have been modified.

The chief difficulty in working with DOPA is its well-known ease of oxidation probably to the quinone, and other products, and this formed the basis for protection of the phenolic groups. A variety of methods have been used to prepare O-protected DOPA, which are amenable to routine peptide synthesis (CHART B.18).[27] A strategy found effective for the prevention of premature DOPA metabolism (*vide supra*) is to systematically block the relevant sites, namely, the COOH, NH_2 and catechol systems. An exhaustive study with synthetic analogs, with various combinations have shown that several of the compounds described in CHART B.19, effectively prevent premature DOPA metabolism and therefore better bioavailability of the drug.[24]

L-DOPA has been used in the chiral synthesis of several natural products. Noteworthy among these is the synthesis of (*S*)-reticuline, the preparation of which required specifically 4-O-benzyl protected DOPA. A study of the action of benzyl halides with N,C- protected DOPA showed the formation of mixtures. When EtOH was used as a solvent trans esterification took place. The desired 4-benzyl protected DOPA was formed in 27% yields form N-formyl-DOPA methyl ester by reaction with K_2CO_3 in DMSO (CHART B.20).[8] As stated previously (CHART B.6), 4-benzyl-N,C- protected DOPA has been made from tyrosine.[9]

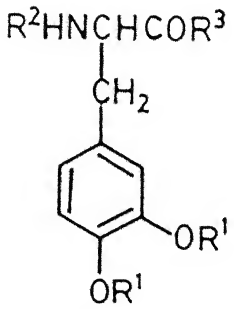
More recently, selective pivaloyl protection of 4-hydroxyl group has been reported (CHART B.21).[28,29] The presence of the catechol unit in DOPA has made DOPA crown ethers as attractive synthetic targets. N,C- protected DOPA affords crown ethers on alkylation with oligo (ethylene glycol) dibromides.[30] Sequential deprotection and EEDQ mediated peptide bond formation afforded DOPA-DOPA crown complexes, which was transformed to either diketo piperazine (CHART B.22)[31] or polymers. These crown ether derivatives complex K^+, NH_4^+, $LeuH^+$ and $GlyH.^+$ They are also phase transfer

CHART B.18



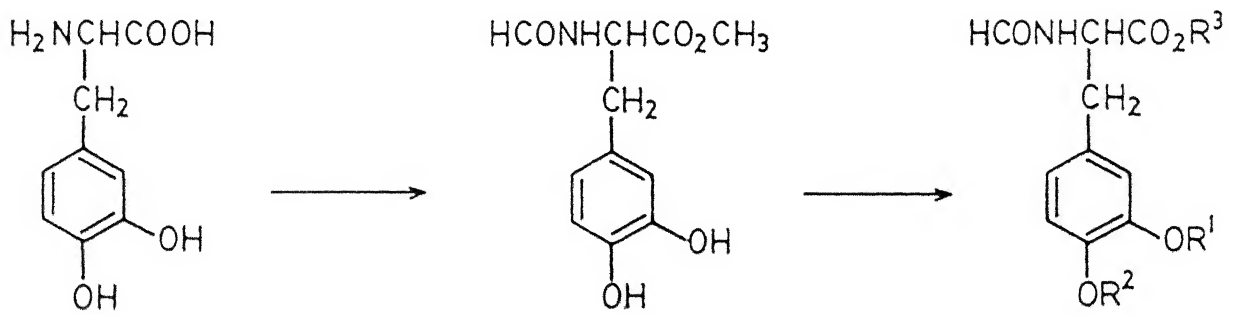
R	R'	R"
H	H	OH
$COCH_3$	Boc	$OC_6H_4NO_2$
$CH_2C_6H_5$	Boc	$OC_6H_4NO_2$
H	H	$OCH_3 \cdot HCl$
H	Boc	OCH_3
H	Boc	OH
$COCH_3$	Boc	OH
$CH_2C_6H_5$	Boc	OH

CHART B.19



R^1	R^2	R^3
CH_3CO	H·HCl	OH
$(CH_3)_3CCO$	H·HCl	OH
$(CH_3)_3CCO$	HCO	OK
H	H·HCl	OCH_3
CH_3CO	H·HCl	OCH_3
CH_3CO	H·HCl	$OCH_2C_6H_5$
H	H·HCl	$OCH_2C_6H_5$
CH_3CO	$HCl \cdot NH_2CH_2CO$	OH
CH_3CO	$HCl \cdot NH_2CH_2CO$	OCH_3
CH_3CO	H·HCl	$NHCH_2COOH$

CHART B.20



R_1	R_2	R_3
H	$PhCH_2$	Et
$PhCH_2$	H	Et
H	$PhCH_2$	Me
$PhCH_2$	H	Me

CHART B.21

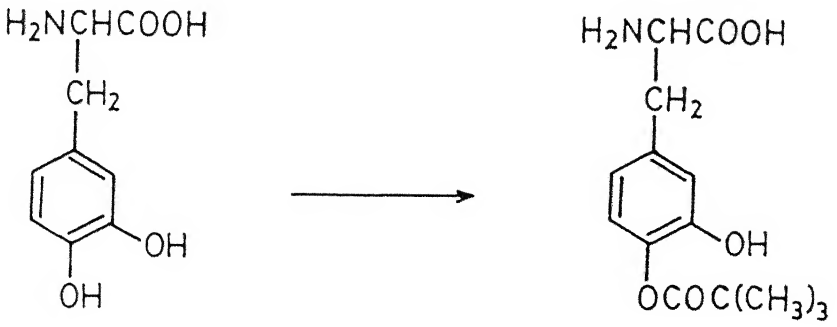
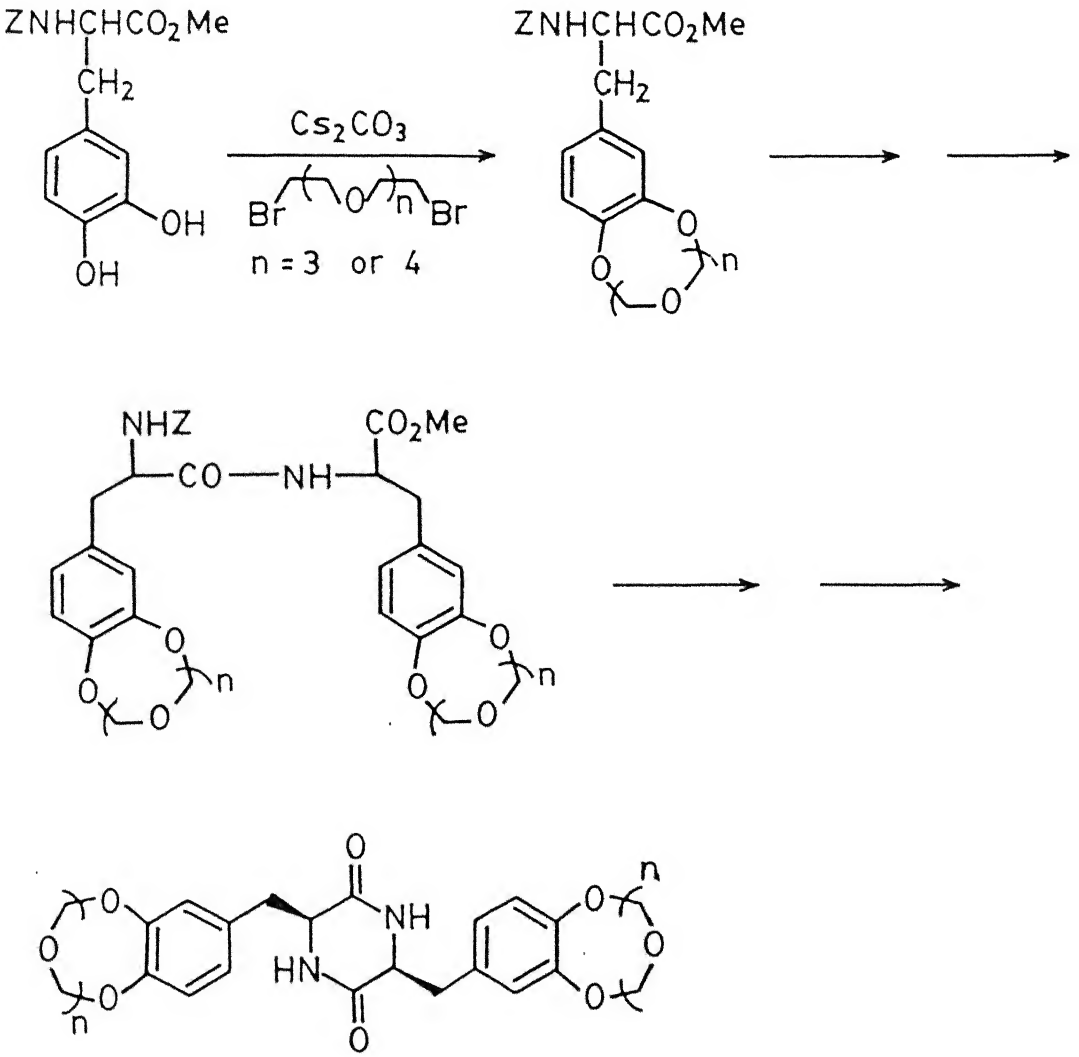


CHART B.22



reagents.[31]

The catecholic amino acid 5-S-cysteinyl-3,4-dihydroxyphenylalanine (5-S-cysteinyl-DOPA) is the chief building stone of pheomelanins, yellow to reddish-brown melanins. Reaction of L-DOPA with L-cystine in boiling aqueous 47% HBr gave 1.2% of 5-S-cysteinyl DOPA. However the compound could be obtained in approximately 70% yields by an oxidation - addition sequence (CHART B.23).[15]

Protected form of fluorescent chromophore of the siderophore pseudobactin has been prepared from DOPA, indicating the involvement of DOPA in the biosynthesis of siderophores from pseudomonas (CHART B.24).[32]

A series of di- and tri-peptides containing L-DOPA have been synthesized and examined for anti-Parkinson activity. Interestingly, some of the compounds were effective in reversing reserpine induced catalomia than L-DOPA (CHART B.25).[24,33]

Di-OAc-DOPA has been transformed to N-carboxy anhydride and polymerized (CHART B.26).[34] Such polymers have many potential uses, such as formation of quinones. Such polymers show a solvent dependent helical sense.[35]

CHART B.23

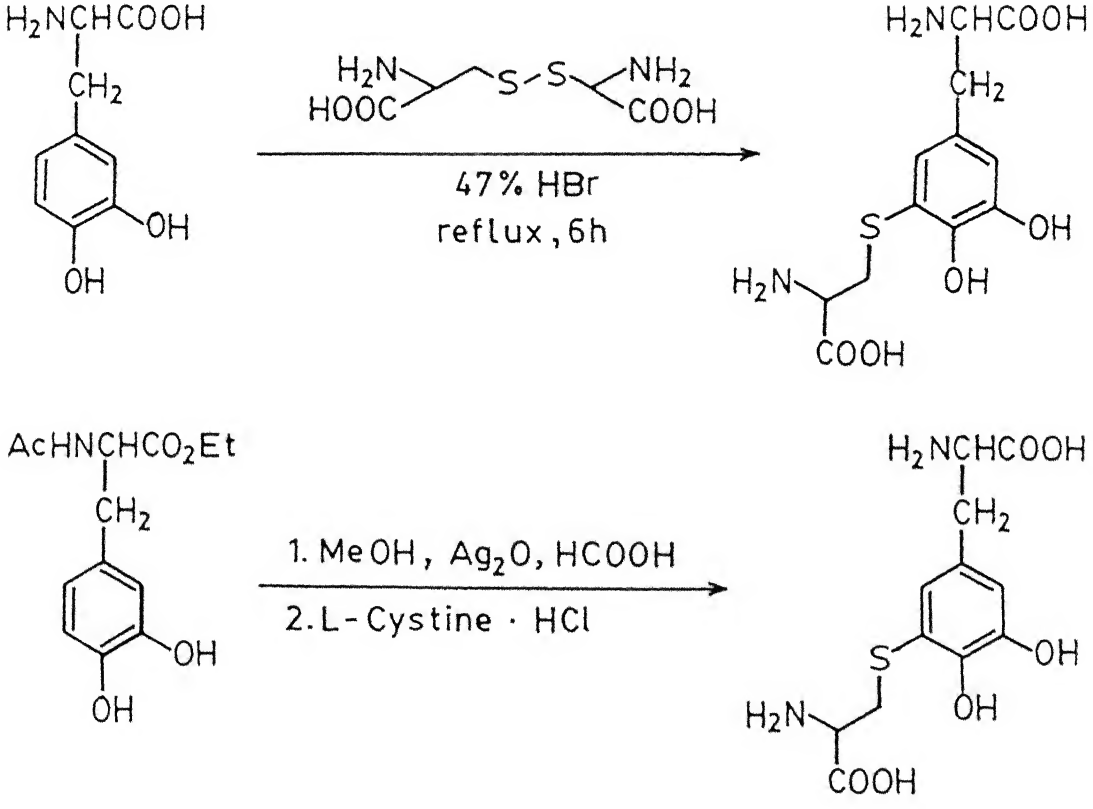

H2NCHCOOH
CH2
OH
OH
H2N
HOOC
S
S
NH2
COOH
47% HBr
reflux, 6h
H2NCHCOOH
CH2
S
OH
H2N
OH
COOH
AcHNCHCO2Et
CH2
OH
OH
1. MeOH, Ag2O, HCOOH
2. L-Cystine · HCl
H2NCHCOOH
CH2
S
OH
H2N
OH
COOH

CHART B.24

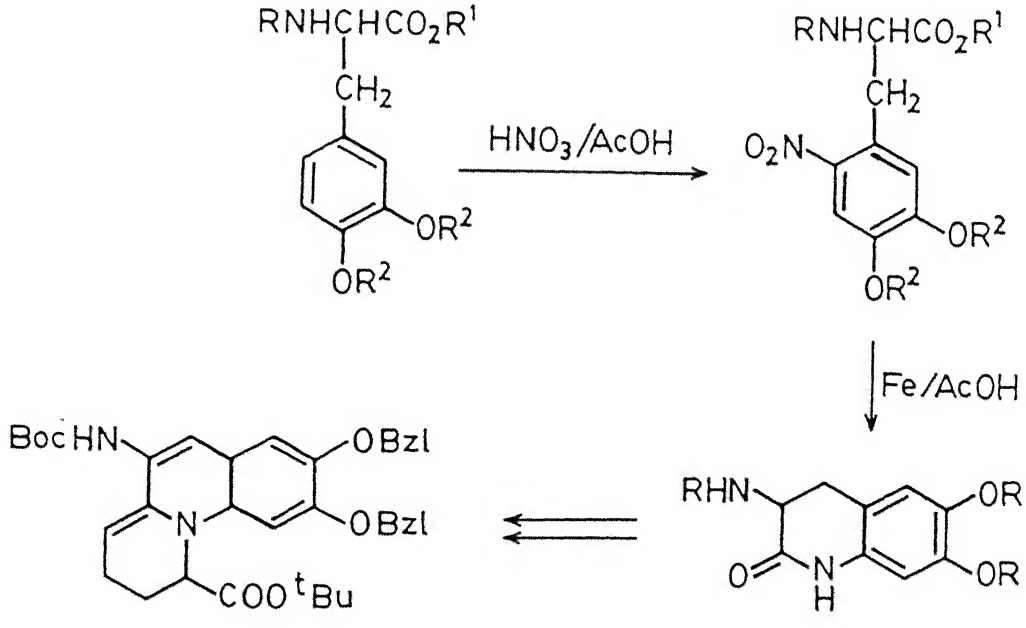

RNHCHCO2R1
CH2
OR2
OR2
HNO3/AcOH
O2N
Fe/AcOH
RHN
OR
O
N
H
BocHN
OBzl
N
COO tBu
R =
R1 = Me
R2 = -CH2Ph (Bzl)

CHART B.25

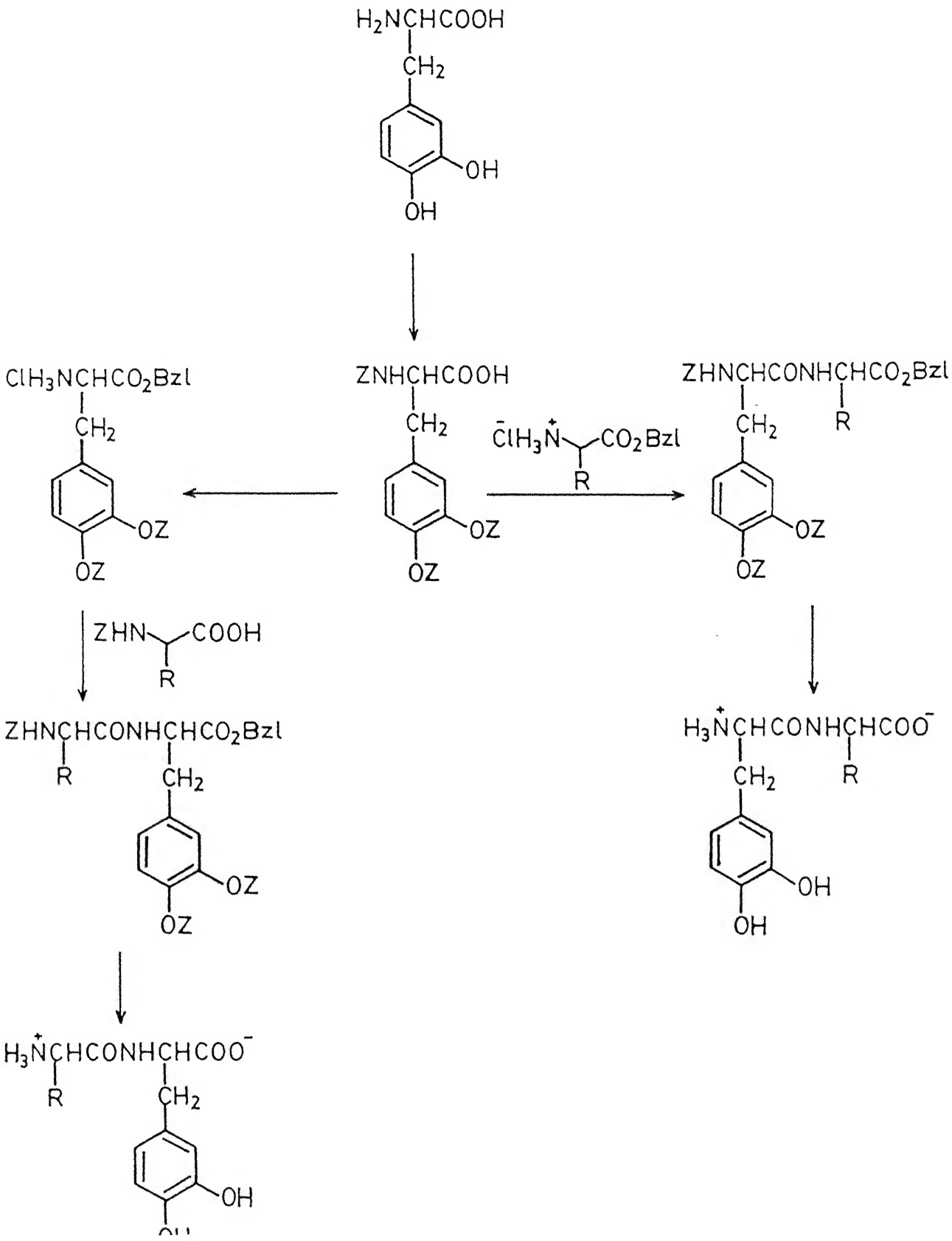
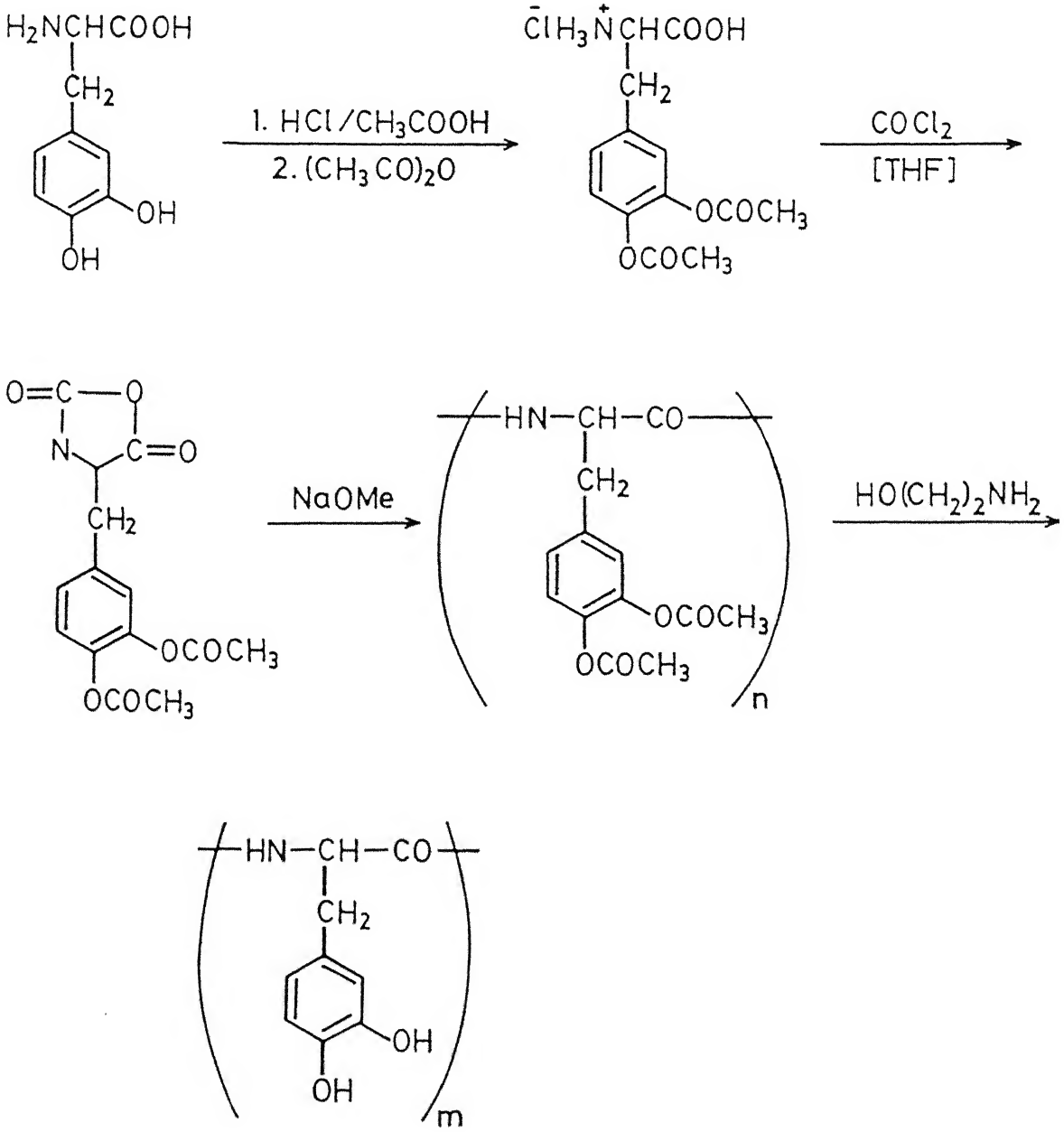

H2NCHCOOH
CH2
OH
OH
ClH3NCHCO2Bzl
ZNHCHCOOH
ZHNCHCONHCHCO2Bzl
R
OZ
ZHN
COOH
H3NCHCONHCHCOO
CH2

CHART B.26

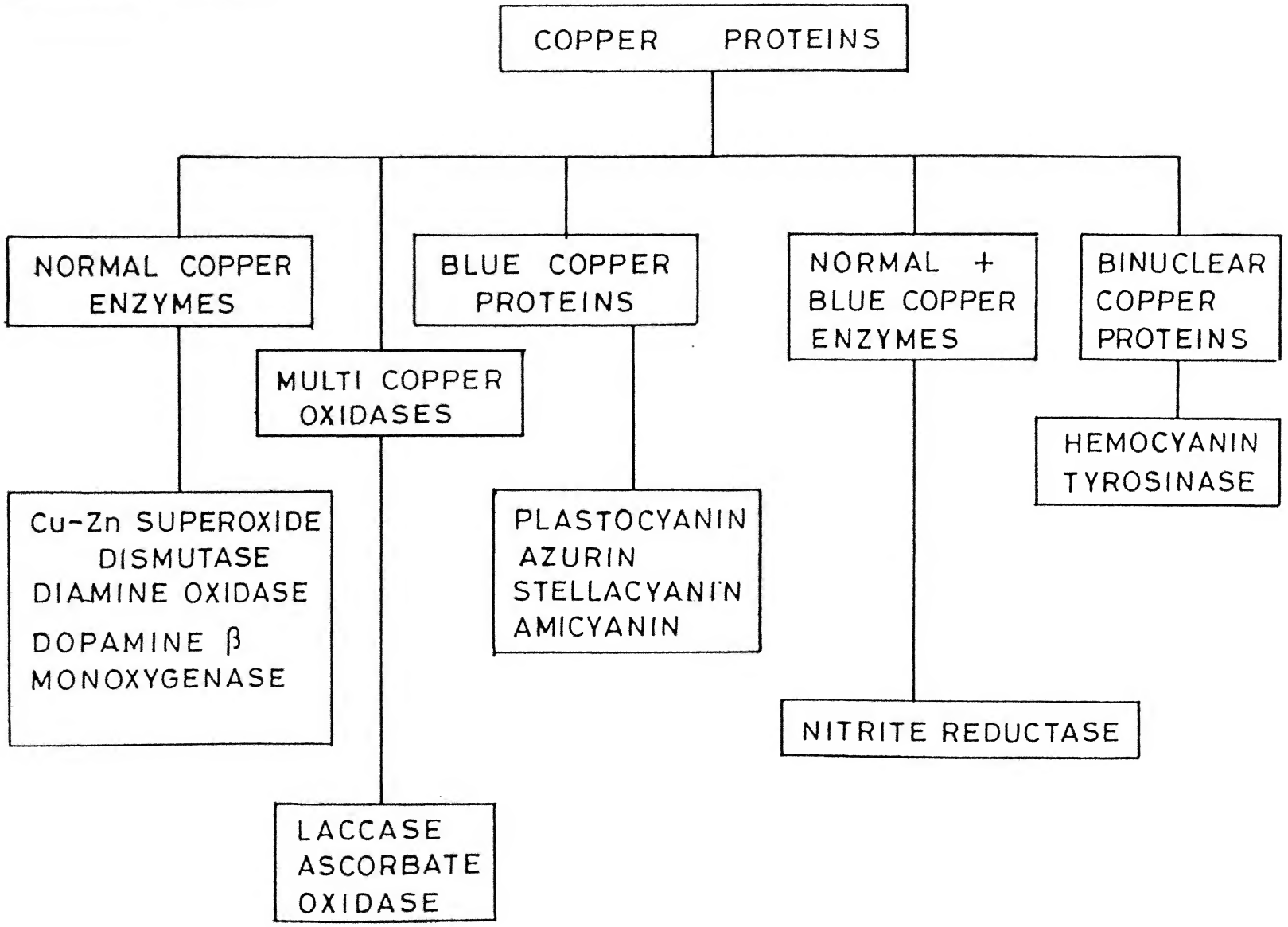

H2NCHCOOH
CH2
OH
OH
1. HCl/CH3COOH
2. (CH3CO)2O
ClH3NCHCOOH
CH2
OCOCH3
OCOCH3
COCl2
[THF]
O=C—O
N
C=O
CH2
OCOCH3
OCOCH3
NaOMe
HN—CH—CO
CH2
OCOCH3
OCOCH3
n
HO(CH2)2NH2
HN—CH—CO
CH2
OH
OH
m

C. PRESENT WORK

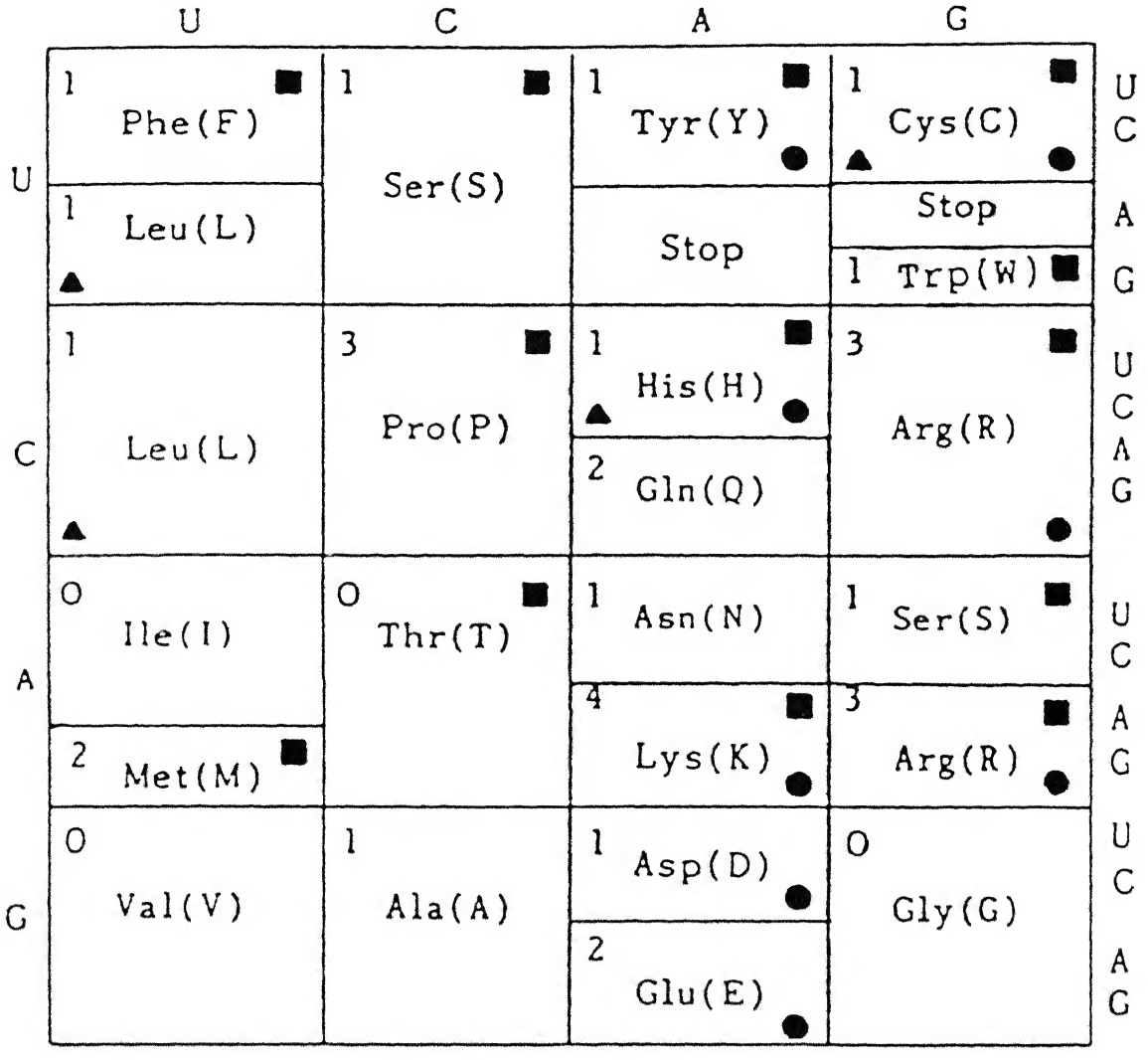
C.I. THE CRAFTING OF PEPTIDE SEGMENTS WITH Cu(II) UPTAKE POTENTIAL

The interaction of oxygen with copper centered co-ordination spheres nestled in the cradle, crafted from the protein manifold, brings about key biological transformations. Such manifestations are seen, *inter alia*, in, normal copper enzymes associated with dismutation of superoxide radical, oxidation of amines, oxidation of hydroxy functions and hydroxylations, blue copper proteins involved in outer sphere long range electron transfer, hemocyanins that reversibly bind oxygen, bi-nuclear copper proteins associated with oxygen transport and aromatic hydroxylations and the multi-copper oxidases that couple four one electron oxidations of substrates to the reduction of oxygen to water.[36] Thus the general profile of copper proteins is presented in SCHEME C.I.1.[37] The genetic code which correlates the information system with the functional one is presented in SCHEME C.I.2. In spite of the fact that infinite number of α-amino acid structures could be conceived and the fact that over 1000 such compounds do occur in Nature, the choice of 20 amino acids into the code complement should reflect a very high degree of selection. This is attested by the fact that barring minor aberrations the code is functional across the living domain. A structural examination of the nature of the side chains of the 20 coded amino acids should reveal a high degree of versatility pertaining to the creation of unique environments which are characteristic of the functional enzymes. Mostly this notion is well justified. Important exceptions do exist, one of which being the inability of any of the coded amino acid side chains to have independent copper uptake potential. Although the focus of the present work pertains to Cu(II) uptake, it must be pointed out that none of the coded amino acid side chains has independent metal uptake property. This is rather surprising since metal ions play a pivotal role in enzyme

SCHEME C.I.1


COPPER PROTEINS
NORMAL COPPER ENZYMES
BLUE COPPER PROTEINS
NORMAL + BLUE COPPER ENZYMES
BINUCLEAR COPPER PROTEINS
MULTI COPPER OXIDASES
HEMOCYANIN
TYROSINASE
Cu-Zn SUPEROXIDE DISMUTASE
DIAMINE OXIDASE
DOPAMINE β MONOXYGENASE
PLASTOCYANIN
AZURIN
STELLACYANIN
AMICYANIN
NITRITE REDUCTASE
LACCASE
ASCORBATE OXIDASE

SCHEME C.I.2



The Genetic Code

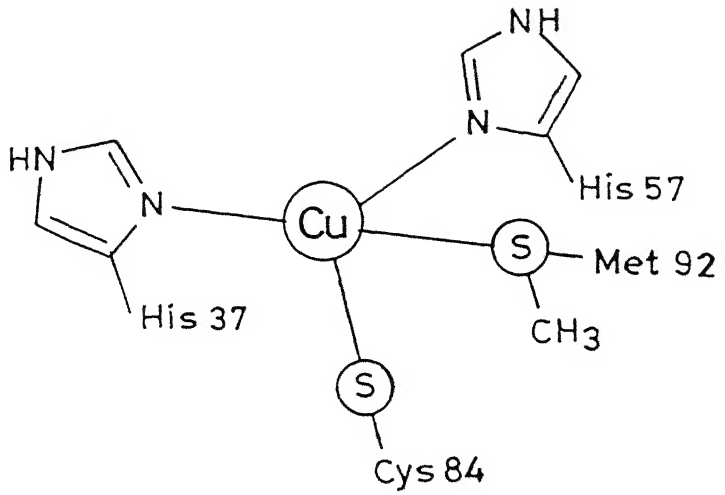
common metal configuration observed in enzymes pertains to redox operations. Such metallo enzymes are characterised by the presence of a substrate binding domain as well as a metal binding region. The metal binding regions, which are active sites in the redox processes, are considered as autonomous entities. That is such regions when transplanted to other systems are likely to acquire metal uptake potential. With respect to copper co-ordination, the metal co-ordination is largely accomplished by the careful placement of a handful of coded amino acids, notable amongst which are histidine, tyrosine, aspartic acid, cysteine and methionine. This aspect is illustrated in SCHEME C.I.3, SCHEME C.I.4, SCHEME C.I.5 and SCHEME C.I.6. In SCHEME C.I.3. is shown the copper binding site in plastocyanin.[38] The copper is co-ordinated to pairs of histidines and to methionine and cysteine. Here the peptide segment that harbors the metal ion spans from His-37 to Met-92, thus involving 55 residues.

The profile of the copper binding site in azurin (SCHEME C.I.4)[39] involves Glu-45, His-46, Cys-112, His-117 and Met-121, thus representing a span of 76 residues.

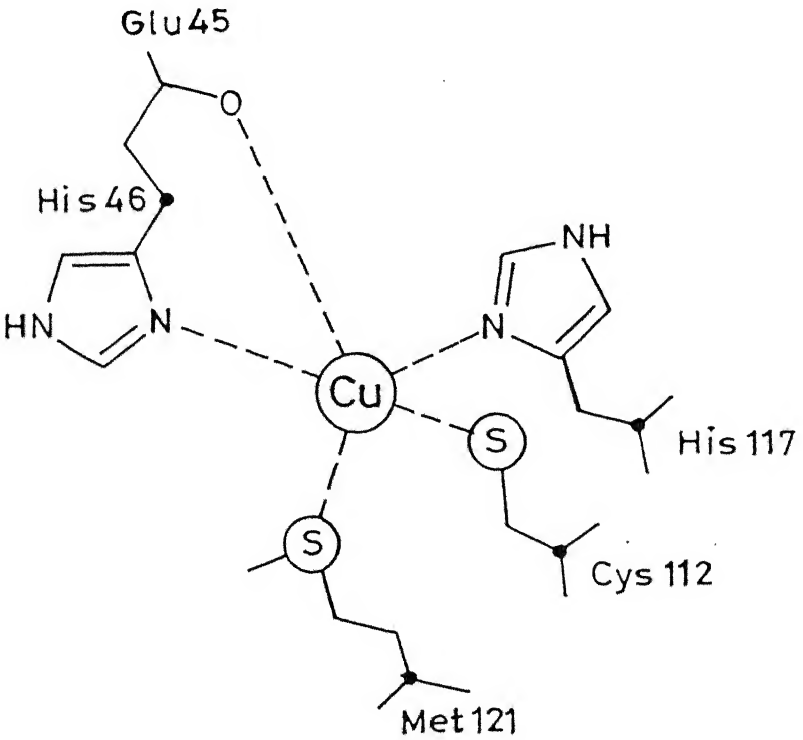
It is obvious that when such metallo enzymes bind the substrates the latter and the active sites should be in close proximity allowing the operation of the redox processes. The substrate binding site being necessarily of specific nature would require a stretch of residues for its proper construction. Recent X-ray crystallographic studies have pin pointed the metal active sites and the substrate binding sites in metallo enzymes. It is logical to assume that where a metal binding site is placed in close proximity to the substrate binding site the expected redox process would ensue.

The relationship between substrate binding site and the metal binding site is illustrated in SCHEME C.I.5 pertaining to galactose oxi dase.[40] One could see here that the bound substrate is placed in close proximity to the metal center, thus promoting ready oxidation.

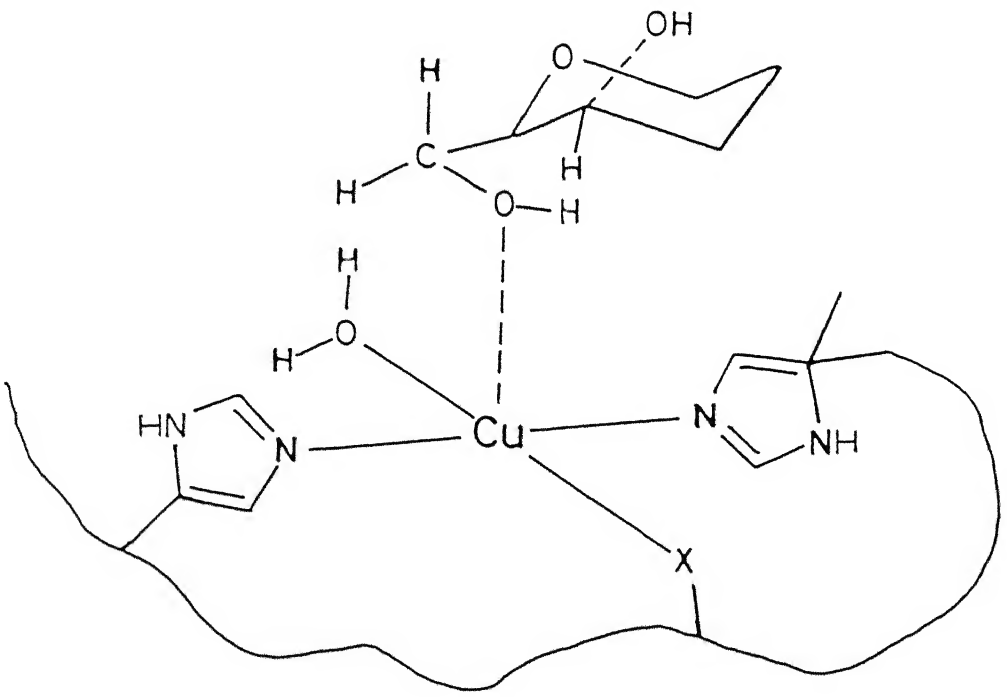
SCHEME C.I.3



SCHEME C.I.4



SCHEME C.I.5



The situation could become even more complex when the active metal center is crafted making use of a template metal. This is illustrated in SCHEME C.I.6 with the binding site in copper superoxide dismutase,[41] where the active copper ion is nestled within the confines of four imidazoles whose specific alignment is promoted by neighbouring tetrahedrally co-ordinated non redox active Zn(II) template.

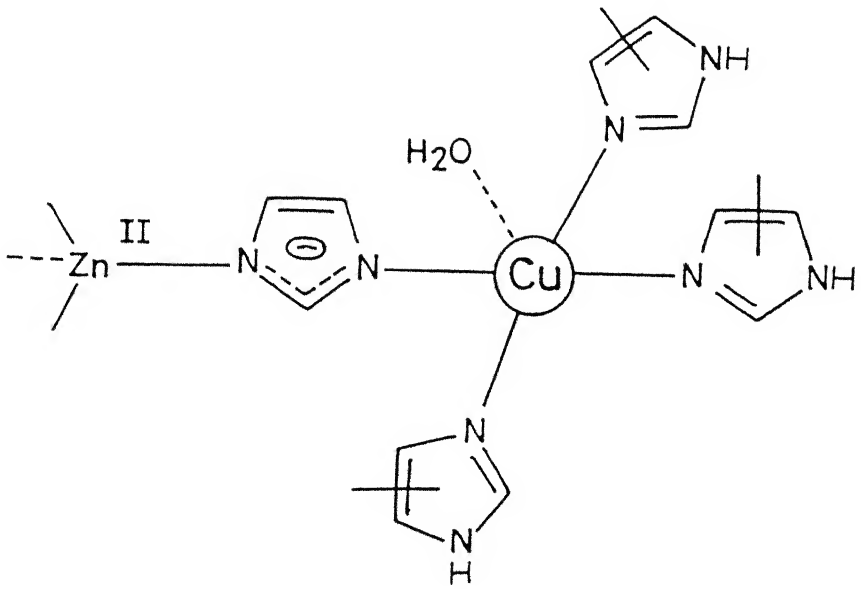
The successful transformation of proteolytic enzyme papain to 'flavo papain' by placement of a redox active unit close to the substrate binding site[42] made it highly logical to anticipate that when a metal binding site can be crafted in close proximity to the substrate binding site of a redox active metallo protein, the expected metallo enzyme activity would be observed. This would amount to 'a minimalistic designed approach' to metallo enzyme mimics wherein the necessary metal ion would be placed on the side chain of single residue rather than a combination that would require a stretch of sequences.

The present work reports the first synthesis of modified tyrosine analogs which have independent copper(II) uptake potential and which can be incorporated into peptide segments by the usual procedures.

The reaction of L-tyrosine (Tyr) with AcCl-$AlCl_3$ in nitrobenzene at 100°C for 6 h afforded 3-acetyl-tyrosine-hydrochloride [Tyr(3-Ac).HCl] (1) in 69% yields. Esterification of (1) with methanolic HCl afforded Tyr(3-Ac)OMe.HCl (2). Compound (2) was N-protected with BzCl-Na_2CO_3 to provide BzTyr(3-Ac)OMe (3).

During literature survey an unusual coincidence was observed namely Tyr(3-Ac).HCl (1) and its isomer Tyr(O-Ac).HCl are reported[43] are to have the same melting point of 223°C. Although the NMR spectrum of (1) is totally in agreement with the structure assigned, coupled with the fact that (1) has been transformed to DOPA with alkaline H_2O_2, it was felt prudent to make a direct comparison of the isomers. With this in

SCHEME C.I.6



view BzTyr(O-Ac)OMe (3a) was prepared from tyrosine via esterification (MeOH-HCl), N-benzoylation (BzCl-Na_2CO_3) and acetylation (Ac_2O-Py). As expected, compound (3) (mp 131-133°C) and compound (3a) (mp 118° C) were quite different and their spectral properties (NMR and IR) were in excellent agreement with their proposed structures.

Tyr(3-Ac)OMe (2) was transformed to ZTyr(3-Ac)OMe (4) (ZCl-Na_2CO_3). Treatment of compound (2) with Ac_2O and pyridine afforded compound (5), wherein, both the phenolic and the amino function underwent acetylation. Treatment of (5) with aq.$NaHCO_3$-MeOH gave AcTyr(3-Ac)OMe (6) (CHART C.I.1).

---

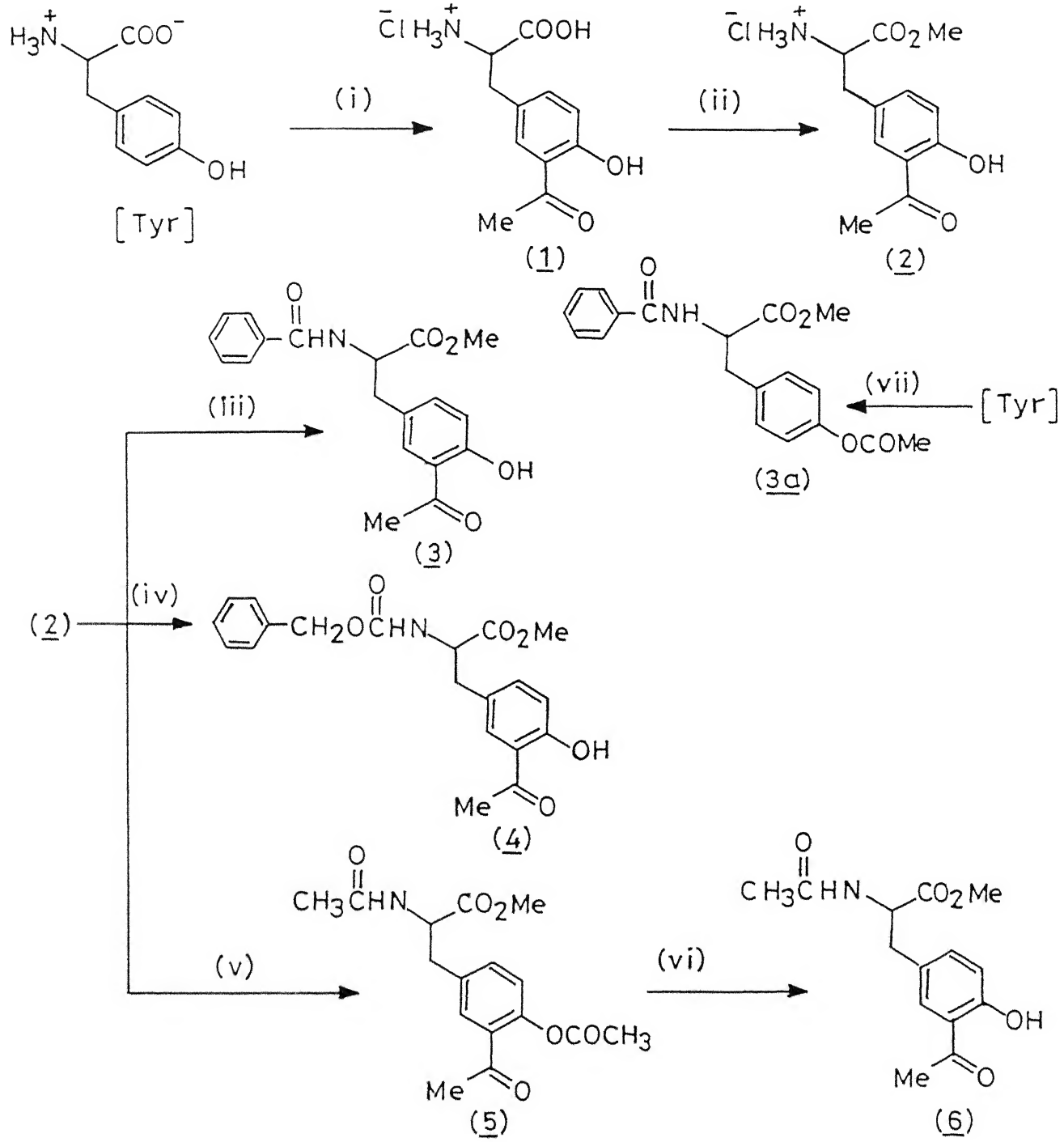
(1)

yield	:	79%
mp	:	220-223°C (dec.) (lit.[9] mp 220-224°C)
ir(KBr)ν_{max} cm^{-1}	:	3000 (br), 1742, 1640, 1582, 1518, 1499
nmr(D_2O) δ	:	2.3 (s, 3H, CH_3), 3.0 (d, 2H, $C^{\beta}H_2$), 4.12 (t, 1H, $C^{\alpha}H$), 6.71 (d, 1H, Tyr C-5 H), 7.26 (d, 1H, Tyr C-6 H), 7.54 (s, 1H, Tyr C-2 H)
ms (m/z)	:	224 $(MH)^+$-HCl
$[\alpha]_D^{25}$	:	-2.33° (c, 1.0, MeOH)

(2)

yield	:	99%
mp	:	186-187°C (dec.) (lit.[9] mp 180-183°C)
ir(KBr)ν_{max} cm^{-1}	:	3428, 2923, 1751, 1637, 1490
nmr(D_2O) δ	:	2.5 (s, 3H, CH_3), 3.12 (d, 2H, $C^{\beta}H_2$), 3.72 (s, 3H, $COOCH_3$), 6.87 (d, 1H, Tyr C-5 H), 7.3 (d, 1H, Tyr C-6H), 7.67 (s, 1H, Tyr C-2 H)
ms (m/z)	:	238 $(MH)^+$-HCl
$[\alpha]_D^{25}$	:	-3.43° (c, 0.3, MeOH)

CHART C.I.1



(i) AcCl, $AlCl_3$/$PhNO_2$, 100°, 6h (ii) MeOH–HCl

(iii) Bz–Cl, Na_2CO_3 / H_2O–Et_2O (iv) Z–Cl, Na_2CO_3 / H_2O–Et_2O

(v) Ac_2O, Py (vi) aq. $NaHCO_3$, MeOH

(vii) ⓐ MeOH–HCl ⓑ BzCl, Na_2CO_3 / H_2O–Et_2O ⓒ Ac_2O, Py

(3)

yield	:	86%
mp	:	131-133°C
ir(KBr)ν_{max} cm^{-1}	:	3443, 3068, 1750, 1637, 1580, 1517
nmr($CDCl_3$) δ	:	2.51 (s, 3H, CH_3), 3.31 (d, 2H, $C^{\beta}H_2$), 3.84 (s, 3H, $COOCH_3$), 5.12 (dd, 1H, $C^{\alpha}H$), 6.65 (d, 1H, NH), 6.87-7.93 (m, 8H, aromatic), 12.19 (s, 1H, OH)
ms (m/z)	:	342 $(MH)^+$

(3a)

yield	:	83%
mp	:	118°C
ir(KBr)ν_{max} cm^{-1}	:	3317, 1740, 1639, 1512
nmr($CDCl_3$) δ	:	2.34 (s, 3H, $OCOCH_3$), 3.31 (d, 2H, $C^{\beta}H_2$), 3.81 (s, 3H, $COOCH_3$), 5.12 (d, d, 1H, $C^{\alpha}H$), 6.75 (d, 1H, NH), 6.93-7.90 (m, 9H, aromatic)

(4)

yield	:	98%
mp	:	93-95°C (lit.[9] mp 94-96°C)
ir(KBr)ν_{max} cm^{-1}	:	3308, 1745, 1685, 1642, 1541
nmr($CDCl_3$) δ	:	2.53 (s, 3H, CH_3), 3.13 (m, 2H, $C^{\beta}H_2$), 3.78 (s, 3H, $COOCH_3$), 4.63 (dd, 1H, $C^{\alpha}H$), 5.13 (s, 2H, Z CH_2), 5.31 (d, 1H, NH), 6.81-7.63 (m, 8H, aromatic), 12.16 (s, 1H, OH)
ms (m/z)	:	372 $(MH)^+$

(5)

yield	:	80%
mp	:	96°C
ir(KBr)ν_{max} cm^{-1}	:	3306, 2954, 1751, 1685, 1647, 1580, 1548
nmr($CDCl_3$) δ	:	2.0 (s, 3H, $NCOCH_3$), 2.34 (s, 3H, $OCOCH_3$), 2.53 (s, 3H, $COCH_3$), 3.19 (d, 2H, $C^{\beta}H_2$), 3.78 (s, 3H, $COOCH_3$), 4.91 (dd, 1H, $C^{\alpha}H$), 6.19 (d, 1H, NH), 6.97-7.63 (m, 3H, aromatic)
ms (m/z)	:	322 $(MH)^+$-HCl

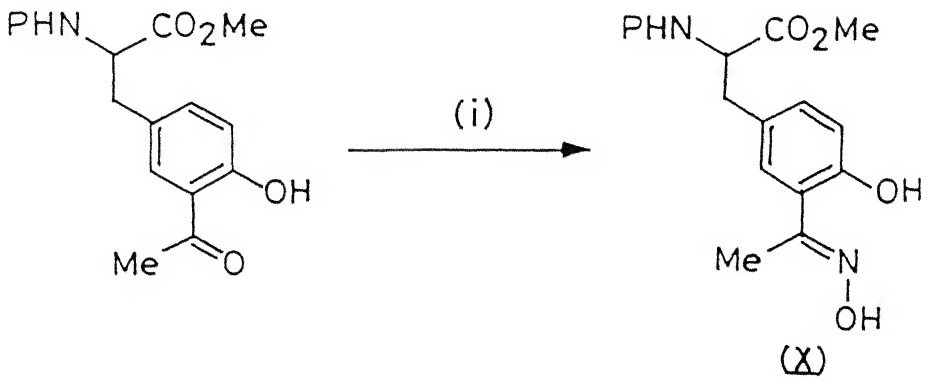
(6)

yield	:	83%
mp	:	136°C
ir(KBr)ν_{max} cm^{-1}	:	3299, 3070, 1733, 1650, 1595, 1544
nmr($CDCl_3$) δ	:	2.03 (s, 3H, $NCOCH_3$), 2.63 (s, 3H, $COCH_3$), 3.16 (d, 2H, $C^{\beta}H_2$), 3.81 (s, 3H, $COOCH_3$), 4.91 (d, 1H, $C^{\alpha}H$), 6.0 (d, 1H, NH), 6.97 (d, 1H, Tyr C-5 H), 7.25 (d, 1H, Tyr C-6 H), 7.53 (s, 1H, Tyr C-2 H), 12.16 (s, 1H, OH)
ms (m/z)	:	280 $(MH)^+$

In principle, compounds (3), (4) and (6) having a proximate alignment of the hydroxyl and the acetyl groups should exhibit a profile similar to o-hydroxy acetophenone. Indeed the appearance of the hydroxyl protons in these cases at about 12 ppm clearly indicates that it is strongly hydrogen bonded to the carbonyl function. It was anticipated, therefore, these compounds would readily form complexes with metal ions, particularly Cu(II). This would have made the simple Tyr(3-Ac) side chain as one possessing metal uptake potential. However, all efforts to prepare copper complexes of (3), (4) and (6) failed, thus necessitating further modifications. These endeavours, *vide infra*, resulted in substrates having ready copper uptake potential and having three types of structural profile. These are, respectively, the oximes, the Schiff bases derived from acetylacetone - ethylenediamine (AEH) mono-Schiff base and the bis-Schiff bases derived from ethylenediamine. It should be noted that whereas products derived from AEH involve only a single Tyr(3-Ac) unit, the remaining need two units of the compound.

BzTyr(3-Ac)OMe (3), ZTyr(3-Ac)OMe (4) and AcTyr(3-Ac)OMe (6) smoothly afforded the expected oximes (7), (8) and (9) on treatment with hydroxylamine hydrochloride in aq. $NaHCO_3$-MeOH (CHART C.I.2).

CHART C.I.2



(X)

P	COMPOUND	(X)
Bz	(3)	(7)
Z	(4)	(8)
Ac	(6)	(9)

(i) $NH_2OH \cdot HCl$, $NaHCO_3$ / MeOH

(7)

yield	:	79%
mp	:	172°C
ir(KBr)ν_{max} cm^{-1}	:	3318, 1738, 1706, 1634, 1580, 1526, 1492
nmr($CDCl_3$) δ	:	2.26 (s, 3H, oximino CH_3), 3.2 (d, 2H, $C^{\beta}H_2$), 3.8 (s, 3H, $COOCH_3$), 5.0 (m, 1H, $C^{\alpha}H$), 6.73 -7.93 (m, 9H, NH + aromatic), 11.0 (s, 1H, N-OH), 11.8 (s, 1H, phenolic OH)
ms (m/z)	:	357 $(MH)^+$

(8)

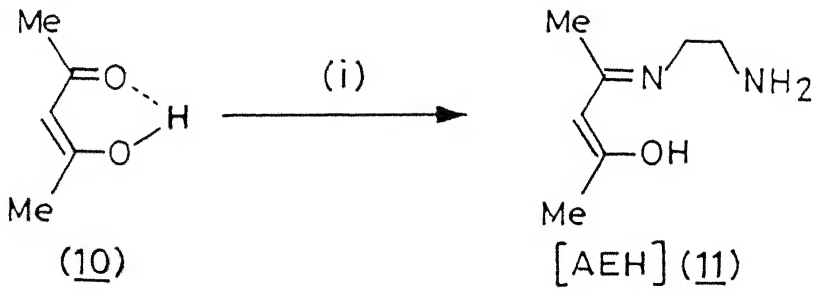
yield	:	91%
mp	:	101-102°C
ir(KBr)ν_{max} cm^{-1}	:	3379, 2922, 2851, 1718, 1686, 1637, 1541
nmr($CDCl_3$) δ	:	2.30 (s, 3H, oximino CH_3), 3.1 (d, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.65 (q, 1H, $C^{\alpha}H$), 5.15 (s, 2H, Z CH_2), 5.5 (d, 1H, NH), 6.9-7.5 (m, 8H, aromatic), 11.6 (s, 1H, N-OH), 12.1 (s, 1H, phenolic OH)
ms (m/z)	:	387 $(MH)^+$

(9)

yield	:	84%
mp	:	135°C
ir(KBr)ν_{max} cm^{-1}	:	3446, 3313, 2931, 1740, 1648, 1535, 1494
nmr($CDCl_3$) δ	:	1.97 (s, 3H, $NCOCH_3$), 2.31 (s, 3H, oximino CH_3), 3.06 (d, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.75 (q, 1H, $C^{\alpha}H$), 6.69-7.19 (m, 4H, NH + aromatic), 10.72 (s, 1H, N-OH), 11.59 (s, 1H, phenolic OH)
ms (m/z)	:	295 $(MH)^+$

Acetylacetone - ethylenediamine mono-Schiff base (AEH) (11) was prepared in 84% yields from acetylacetone (10) with one equivalent of ethylenediamine in chloroform at room temperature (CHART C.I.3).[44]

CHART C.I.3



(10) [AEH] (11)

(i) Ethylenediamine (EDA) / $CHCl_3$, rt , 8h

(11)

yield	:	84%
mp	:	liquid
ir(neat)ν_{max} cm^{-1}	:	3289, 1734, 1609, 1560, 1437, 1325
nmr($CDCl_3$) δ	:	1.66 (m, 2H, NH_2), 2.02 (ss, 6H, CH_3 × 2), 2.9 (m, 2H, CH_2), 3.38 (m, 2H, =N-CH_2), 5.03 (s, 1H, CH), 10.97 (s, 1H, OH)

The NMR spectrum of AEH thus prepared showed complete absence of either ethylenediamine or acetylacetone, which would be the case either when the reaction is incomplete or when the bis Schiff base is formed.

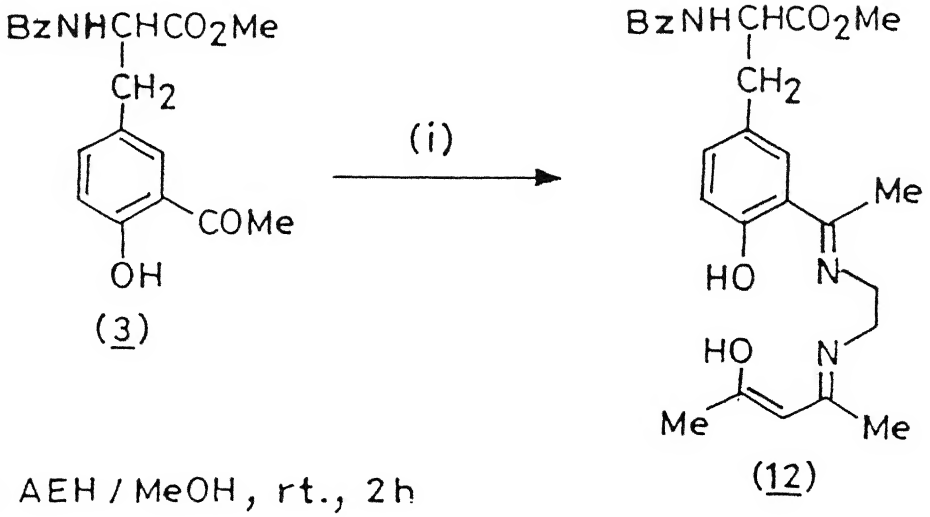
The reaction of BzTyr(3-Ac)OMe (3) with AEH (11) in MeOH at room temperature for 2 h readily afforded the expected Schiff base (12) in 60% yields (CHART C.I.4).

(12)

yield	:	60%
mp	:	190-191°C
ir(KBr)ν_{max} cm^{-1}	:	3273, 3058, 2948, 1747, 1641, 1605, 1582, 1558
nmr($CDCl_3$) δ	:	1.98 (ss, 6H, AEH CH_3 × 2), 2.22 (s, 3H, CH_3), 3.22 (d, 2H, $C^{\beta}H_2$), 3.56-3.97 (m, 7H, -CH_2CH_2- + $COOCH_3$), 5.0 (m, 2H, $C^{\alpha}H$ + enolic-CH), 6.66 (d, 1H, NH), 6.75-7.9 (m, 8H, aromatic), 10.97 (s, 1H, enolic OH), 15.37 (s, 1H, phenolic OH)
ms (m/z)	:	466 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm	:	239, 312

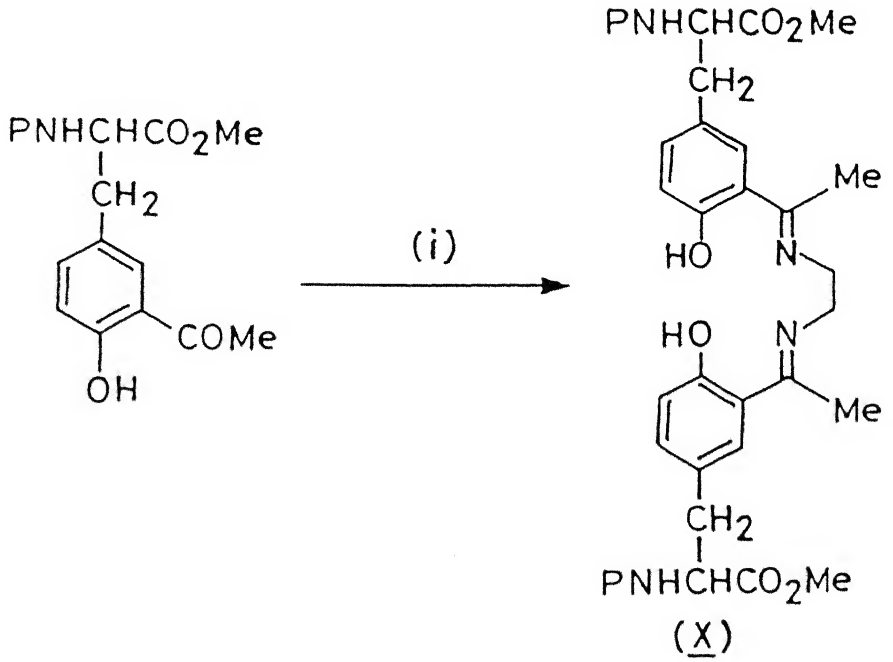
The reaction of BzTyr(3-Ac)OMe (3) and ZTyr(3-Ac)OMe (4) on treatment with methanolic ethylenediamine at room temperature for 12 h afforded the expected Schiff bases (13) and (14), respectively, in 70% and 78% yields (CHART C.I.5).

CHART C.I.4



(i) AEH / MeOH, rt., 2h

CHART C.I.5



P	COMPOUND	(X)
Bz	(3)	(13)
Z	(4)	(14)

(i) Ethylenediamine / MeOH, rt., 12h

(13)

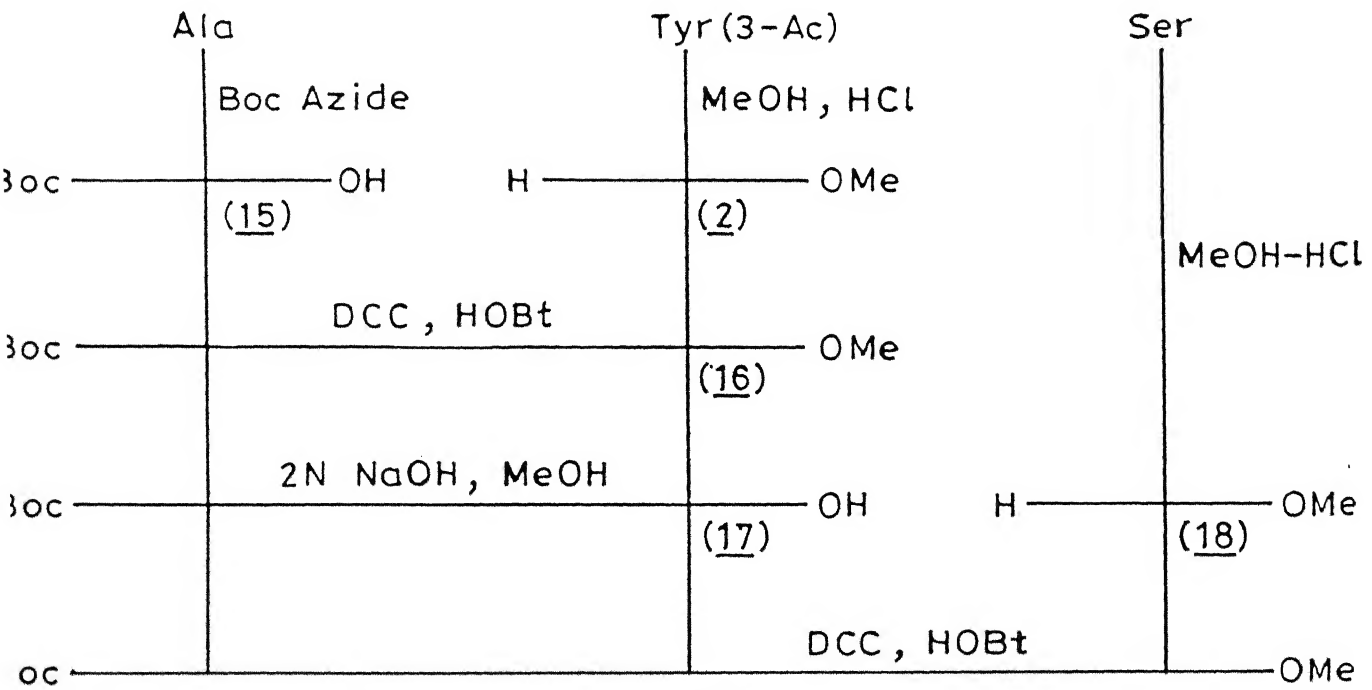
yield	:	70%
mp	:	243-245°C
ir(KBr)ν_{max} cm^{-1}	:	3310, 2953, 1747, 1645, 1632, 1578
nmr($CDCl_3$-TFA)δ	:	2.84 (s, 6H, CH_3 x 2), 3.31 (d, 4H, $C^{\beta}H_2$ x 2), 3.91 (s, 6H, $COOCH_3$ x 2), 4.47 (s, 4H, -CH_2CH_2-), 5.16 (q, 2H, $C^{\alpha}H$ x 2), 7.0-7.88 (m, 16H, aromatic)
ms (m/z)	:	707 $(MH)^+$

(14)

yield	:	82%
mp	:	204°C
ir(KBr)ν_{max} cm^{-1}	:	3412, 1746, 1686, 1619
nmr($CDCl_3$) δ	:	1.37 (s, 6H, CH_3 x 2), 3.15 (d, 4H, $C^{\beta}H_2$ x 2), 3.78 (s, 6H, $COOCH_3$ x 2), 4.0 (s, 4H, -CH_2CH_2-), 4.68 (q, 2H, $C^{\alpha}H$ x 2), 5.18 (s, 4H, Z CH_2 x 2), 5.3 (d, 2H, NH x 2), 6.93-7.53 (m, 16H, aromatic)

The work presented thus far have shown the acetyl unit present in Tyr(3-Ac) can be readily transformed to oximes (7), (8), (9), Schiff base (12) derived from AEH and the bis Schiff bases (13) and (14) derived from ethylenediamine. Work to be presented below would show that these three classes of compounds readily form complexes not only with Cu(II) but also with other similar metal ions. Therefore, the next task was to demonstrate that Tyr(3-Ac) can be incorporated into peptides using normal synthetic methodologies. L-Alanine was N-protected with Boc-azide and the resulting BocAla (15) was smoothly condensed with *in situ* generated Tyr(3-Ac)OMe, derived from (2), using DCC-HOBt procedure in CH_2Cl_2-DMF to afford BocAlaTyr(3-Ac)OMe (16) in 67% yields. Compound (16) was transformed to the corresponding acid (17) (2N NaOH-MeOH) and condensed with *in situ* generated SerOMe, derived from SerOMe.HCl (18) using DCC-HOBt in CH_2Cl_2-DMF to afford BocAlaTyr(3-Ac)SerOMe (19) in 32% yields (CHART C.I.6). The tripeptide (19) has been characterised on the basis of spectral and

CHART C.I.6



Boc Ala Tyr(3-Ac) Ser OMe (19)

analytical data. The ready formation of (19) clearly shows that the non-coded amino acid Tyr(3-Ac) can be easily incorporated into routine peptide synthesis.

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(15)

yield	:	78%
mp	:	80°C (lit.[45] mp 82-84°C)
ir(KBr)ν_{max} cm^{-1}	:	3383, 2989, 1692, 1518, 1456

(16)

yield	:	67%
mp	:	106°C
ir(KBr)ν_{max} cm^{-1}	:	3340, 2982, 1750, 1685, 1651, 1525
nmr($CDCl_3$) δ	:	1.28 (d, 3H, Ala CH_3), 1.37 (s, 9H, Boc CH_3), 2.63 (s, 3H, $COCH_3$), 3.1 (d, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.06 (m, 1H, Ala $C^{\alpha}H$), 4.84 (m, 2H, Tyr $C^{\alpha}H$ + Ala NH), 6.63 (d, 1H, Tyr NH), 6.9 (d, 1H, Tyr C-5 H), 7.22 (d, 1H, Tyr C-6 H), 7.53 (s, 1H, Tyr C-2 H), 11.16 (s, 1H, OH)
ms (m/z)	:	409 $(MH)^+$
$[\alpha]_D^{25}$	:	-5.96° (c, 1.0, MeOH)

(17)

yield	:	98%
mp	:	hygroscopic
ir(KBr)ν_{max} cm^{-1}	:	3349, 2979, 2933, 1716, 1644, 1521, 1488
nmr($CDCl_3$-DMSO-d_6) δ	:	1.35 (d, 3H, Ala CH_3), 1.5 (s, 9H, Boc CH_3), 2.65 (s, 3H, $COCH_3$), 3.1 (d, 2H, $C^{\beta}H_2$), 4.2 (q, 1H, Ala $C^{\alpha}H$), 4.8 (q, 1H, Tyr $C^{\alpha}H$), 5.25 (d, 1H, Ala NH), 6.8 (d, 1H, Tyr NH), 7.0 (d, 1H, Tyr C-5 H), 7.3 (d, 1H, Tyr C-6 H), 7.55 (s, 1H, Tyr C-2 H), 11.6 (s, 1H, phenolic OH)
ms (m/z)	:	395 $(MH)^+$

(18)

yield	:	95%
mp	:	166 °C (lit.[46] mp 166°C)
ir(KBr)ν_{max} cm^{-1}	:	3380, 1730

(19)

yield	:	32%
mp	:	172°C
ir(KBr)ν_{max} cm^{-1}	:	3299, 2931, 1747, 1687, 1647, 1530
nmr($CDCl_3$) δ	:	1.31 (d, 3H, Ala CH_3), 1.47 (s, 9H, Boc CH_3), 2.65 (s, 3H, $COCH_3$), 3.16 (d, 2H, Tyr $C^{\beta}H_2$), 3.65 (s, 3H. $COOCH_3$), 3.81 (d, 2H, Ser $C^{\beta}H_2$), 4.13 (q, 1H, Ala $C^{\alpha}H$), 4.69 (m, 2H, Tyr $C^{\alpha}H$ + Ser $C^{\alpha}H$), 5.03 (d, 1H, Ala NH), 6.78-7.8 (m, 5H, aromatic + Tyr NH + Ser NH), 12.1 (s, 1H, phenolic OH)
ms (m/z)	:	496 $(MH)^+$
$[\alpha]_D^{25}$	:	-21.0° (c, 0.5, MeOH)

Similarly, alanine was Z-protected to (20) (Z-Cl, aq. NaOH) and then condensed with Tyr(3-Ac) using DCC-HOBt procedure in CH_2Cl_2-DMF to afford ZAlaTyr(3-Ac)OMe (21) in 85% yields. Compound (21) was saponified (2N NaOH, MeOH) to (22) and condensed with SerOMe by the same procedure to afford ZAlaTyr(3-Ac)SerOMe (23) in 54% yields (CHART C.I.7).

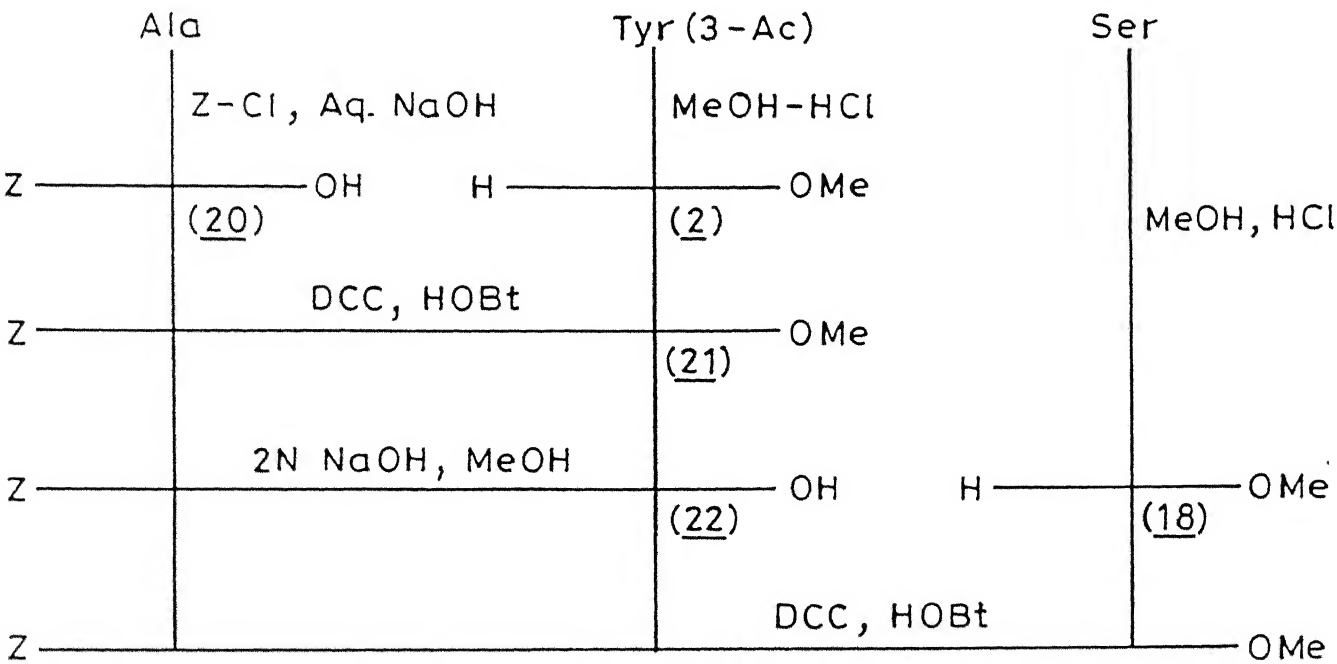
(20)

yield	:	92%
mp	:	96-97°C (lit.[47] mp 97-99°C)
ir(KBr)ν_{max} cm^{-1}	:	3332, 3034, 1701, 1535
nmr($CDCl_3$) δ	:	1.44 (d, 3H, CH_3), 4.38 (m, 1H, $C^{\alpha}H$), 5.16 (s, 2H, Z CH_2), 5.47 (d, 1H, NH), 7.34 (s, 5H, aromatic), 9.69 (s. 1H, COOH)

(21)

yield	:	62%
mp	:	163-164°C
ir(KBr)ν_{max} cm^{-1}	:	3309, 2942, 1741, 1691, 1643, 1526.
nmr($CDCl_3$) δ	:	1.34 (d, 3H, Ala CH_3), 2.59 (s, 3H, $COCH_3$), 3.09 (d, 2H, $C^{\beta}H_2$), 3.78 (s, 3H, $COOCH_3$), 4.19 (m, 1H, Ala $C^{\alpha}H$), 4.84 (q, 1H, Tyr $C^{\alpha}H$), 5.06 (s, 2H, Z CH_2), 5.31 (d, 1H, Ala NH), 6.66 (d, 1H, Tyr NH), 6.88 (d, 1H, Tyr C-5 H), 7.22 (d, 1H, Tyr C-6 H), 7.38 (s, 5H, Z aromatic), 7.53 (s, 1H, Tyr C-2 H), 13.41 (s, 1H, OH)
ms (m/z)	:	443 $(MH)^+$

CHART C.I.7



Z Ala Tyr (3-Ac) Ser OMe (23)

(22)

yield	:	85%
mp	:	184-187°C
ir(KBr)ν_{max} cm^{-1}	:	3282, 1732, 1681, 1640 , 1621, 1525

(23)

yield	:	54%
mp	:	174-176° C
ir(KBr)ν_{max} cm^{-1}	:	3319, 2927, 1743, 1687, 1641, 1538
nmr($CDCl_3$) δ	:	1.34 (d, 3H, Ala CH_3), 2.69 (s, 3H, $COCH_3$), 3.06 (d, 2H, Tyr $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 3.84(d, 2H, Ser $C^{\beta}H_2$), 4.13 (m, 1H, Ala $C^{\alpha}H$), 4.28-4.94 (m, 2H, Tyr $C^{\alpha}H$ + Ser $C^{\alpha}H$), 5.06 (s, 2H, Z CH_2), 6.53 (d, 1H, Ala NH), 6.88 (d, 1H, Tyr C-5 H), 7.09-7.94 (m, 9H, Tyr C-6 H + Tyr C-2 H + Z aromatic + Tyr NH + Ser NH), 12.16 (s, 1H, phenolic OH).
ms (m/z)	:	530 $(MH)^+$

In compounds (19) and (23) the serine residue was located in C-terminal positions. To illustrate that this is of no consequence with respect to incorporation of Tyr(3-Ac) unit ZSerTyr(3-Ac)OMe (25) was prepared in 69% yields by condensation of ZSer (24) and Tyr(3-Ac)OMe by DCC-HOBt procedure in CH_2Cl_2-DMF (CHART C.I.8).

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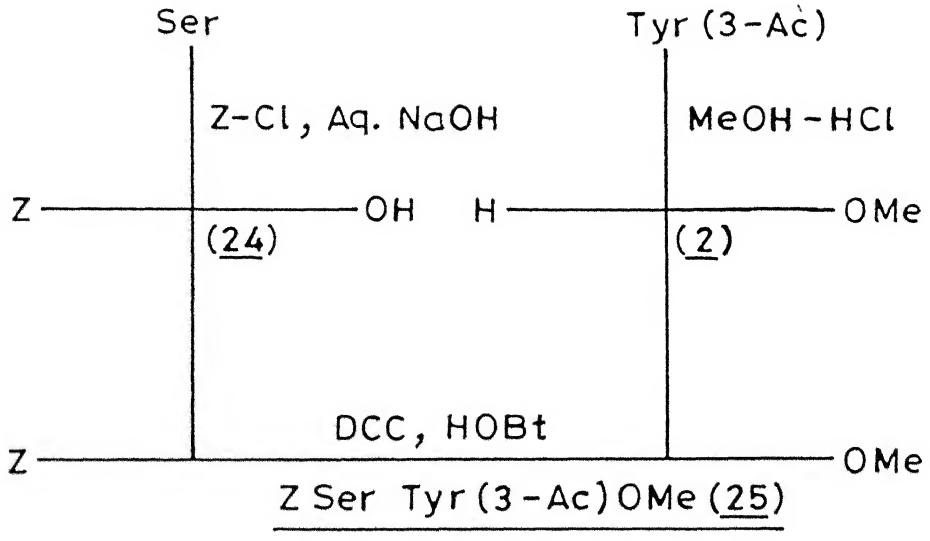
(24)

yield	:	94%
mp	:	115-116°C (lit.[48] mp 114-116°C)
ir(KBr)ν_{max} cm^{-1}	:	3460, 3320, 2928, 1724, 1665, 1515

(25)

yield	:	69%
mp	:	155°C
ir(KBr)ν_{max} cm^{-1}	:	3300, 2926, 1723, 1622, 1511

CHART C.I.8



Z Ser Tyr (3-Ac) OMe (25)

nmr($CDCl_3$-DMSO-d_6) δ	:	2.59 (s, 3H, $COCH_3$), 3.09 (d, 2H, Tyr $C^{\beta}H_2$), 3.72 (m, 5H, $COOCH_3$ + Ser C^{β} H_2), 4.22 (m, 1H, Ser $C^{\alpha}H$), 4.78 (q, 1H, Tyr $C^{\alpha}H$), 5.06 (s, 2H, Z CH_2), 6.53 (d, 1H, Ser NH), 6.88 (d, 1H, Tyr C-5 H), 7.19-7.78 (m, 8H, Z aromatic + Tyr C-6 H + Tyr C-2 H + Tyr NH), 12.16 (s, 1H, phenolic OH).
ms (m/z)	:	459 $(MH)^+$

The smooth transformation of Tyr(3-Ac) unit to the corresponding oxime, in a peptide environment is illustrated in CHART C.I.9. Thus, the dipeptides BocAlaTyr(3-Ac)OMe (16), ZAlaTyr(3-Ac)OMe (21), ZSerTyr(3-Ac)OMe (25) and the tripeptides BocAlaTyr(3-Ac)SerOMe (19) and ZAlaTyr(3-Ac)SerOMe (23), on treatment with hydroxylamine hydrochloride, $NaHCO_3$-MeOH at room temperature afforded the oximes, respectively, (26), (28), (30), (27) and (29) in very good yields (CHART C.I.9).

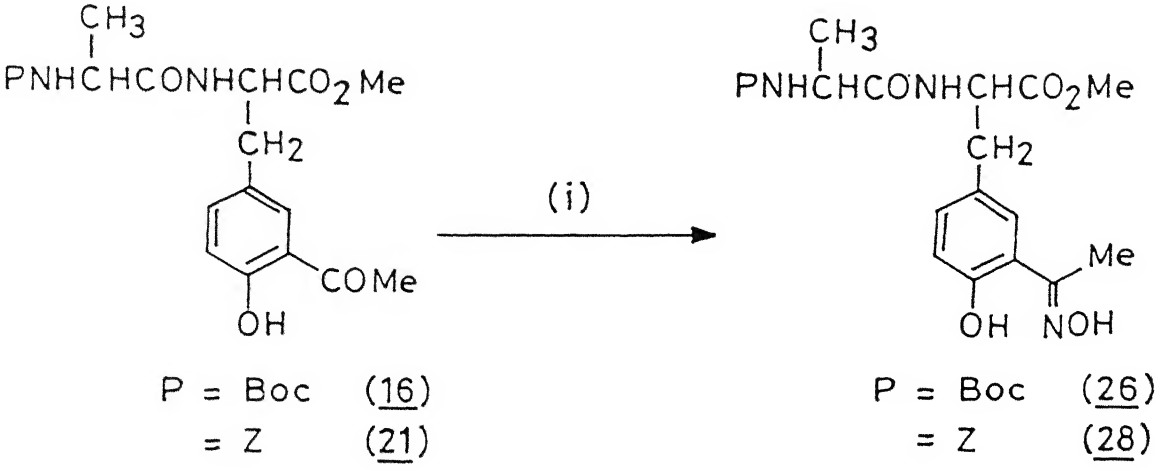
(26)

yield	:	71%
mp	:	172°C
ir(KBr)ν_{max} cm^{-1}	:	3339, 3258,,2986, 2929, 1742, 1690, 1670, 1623, 1537
nmr($CDCl_3$) δ	:	1.47 (m, 12H, Ala CH_3 + Boc CH_3), 2.28 (s, 3H, oximino CH_3), 3.13 (d, 2H, Tyr $C^{\beta}H_2$), 3.84 (s, 3H, $COOCH_3$), 4.17 (m, 1H, Ala $C^{\alpha}H$), 4.93 (q, 1H, Tyr $C^{\alpha}H$), 5.37 (d, 1H, Tyr NH), 6.47-7.34 (m, 4H, aromatic + Tyr NH), 9.43 (s, 1H, N-OH), 11.74 (s, 1H, phenolic OH)
ms (m/z)	:	424 $(MH)^+$

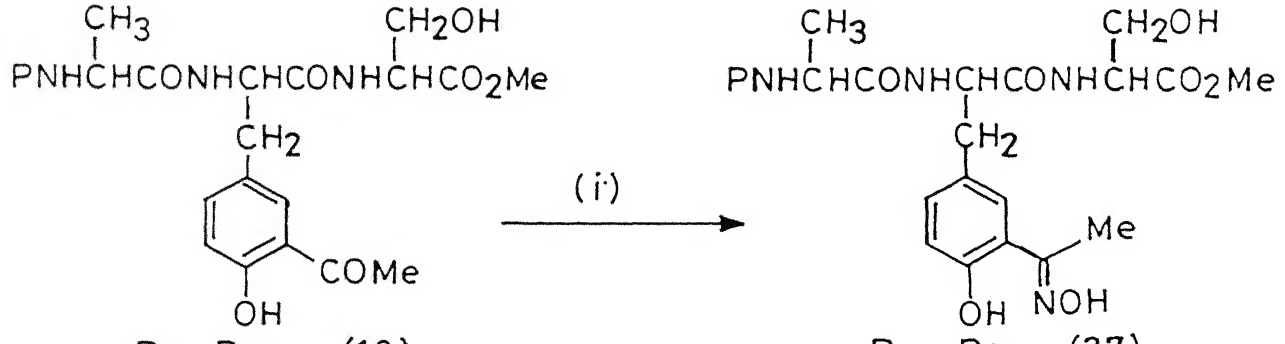
(27)

yield	:	83%
mp	:	104°C
ir(KBr)ν_{max} cm^{-1}	:	3317, 2928, 1743, 1686, 1660, 1521
nmr($CDCl_3$-DMSO-d_6) δ	:	1.28 (d, 3H, Ala CH_3), 1.44 (s, 9H, Boc CH_3), 2.31 (s, 3H, oximino CH_3), 3.09 (d, 2H, Tyr $C^{\beta}H_2$), 3.78 (m, 5H, $COOCH_3$ + Ser $C^{\beta}H_2$), 4.09 (m, 1H, Ala $C^{\alpha}H$), 4.63 (m, 2H, Tyr $C^{\alpha}H$ + Ser $C^{\alpha}H$), 5.75 (d, 1H, Ala NH), 6.75-7.68 (m, 5H, aromatic + Tyr NH + Ser NH), 10.8 (s, 1H, N-OH), 11.7 (s, 1H, phenolic OH)
ms (m/z)	:	511 $(MH)^+$

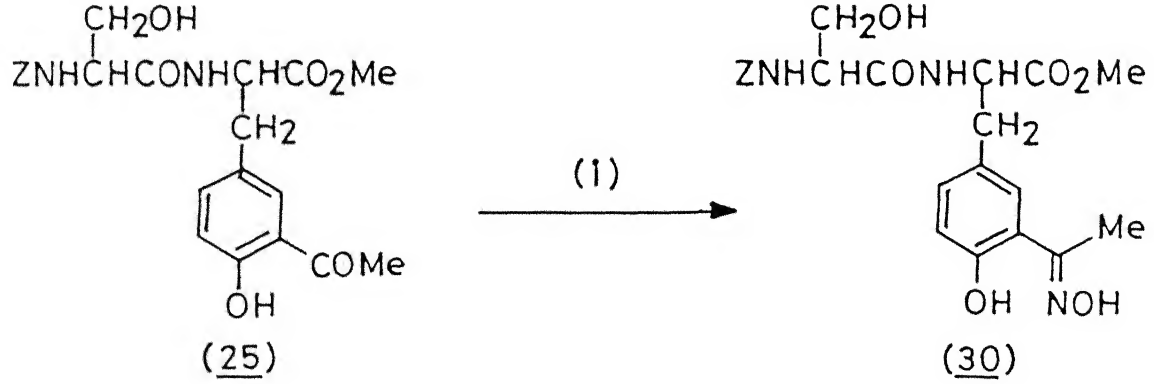
CHART C.I.9



P = Boc (16) P = Boc (26)
= Z (21) = Z (28)



P = Boc (19) P = Boc (27)
= Z (23) = Z (29)



(25) (30)

(i) $NH_2OH \cdot HCl$, $NaHCO_3$ / MeOH

(28)

yield	:	70%
mp	:	165°C
ir(KBr)ν_{max} cm^{-1}	:	3453, 3305, 2929, 1733, 1684, 1650, 1534
nmr($CDCl_3$-DMSO-d_6) δ	:	1.31 (d, 3H, Ala CH_3), 2.31 (s, 3H, oximino CH_3), 3.03 (d, 2H, Tyr $C^{\beta}H_2$), 3.69 (s, 3H, $COOCH_3$), 4.22 (m, 1H, Ala $C^{\alpha}H$), 4.72 (q, 1H, Tyr $C^{\alpha}H$), 5.06 (s, 2H, Z CH_2), 6.50 (d, 1H, Ala NH), 6.75 (d, 1H, Tyr C-5 H), 7.03 (d, 1H, Tyr C-6 H), 7.09-7.63 (m, 7H, Tyr C-2 H + Tyr NH + Z aromatic) 11.06 (s, 1H, N-OH), 11.75 (s, 1H, phenolic OH)
ms (m/z)	:	458 $(MH)^+$

(29)

yield	:	84%
mp	:	204°C
ir(KBr)ν_{max} cm^{-1}	:	3450, 2948, 1720, 1678, 1650
ms (m/z)	:	545 $(MH)^+$

(30)

yield	:	88%
mp	:	145-147°C
ir(KBr)ν_{max} cm^{-1}	:	3527, 3426, 3324, 2945, 1728, 1692, 1645, 1529
ms (m/z)	:	473 $(MH)^+$

Of particular relevance to the objectives of the present work is the efficient condensation of AEH (11) with Tyr(3-Ac) side chains in peptide environment. The importance here is due to the fact that the resulting compounds would readily form metal templates thus enabling the identification of Tyr(3-Ac) as a residue capable of being incorporated into peptides by the usual procedures and at the same time having potential for ready formation of templates that can harbor a range of metal ions. This was experimentally realized. Thus the action of AEH in methanol at room temperature for 2 h readily transforms the dipeptides BocAlaTyr(3-Ac)OMe (16) and ZAlaTyr(3-Ac)OMe (21) to

the corresponding templates (31) and (33) in excellent yields. Similarly, the tripeptide BocAlaTyr(3-Ac)SerOMe (19) afforded template (32) (CHART C.I.10).

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(31)

yield	:	68%
mp	:	163°C
ir(KBr)ν_{max} cm^{-1}	:	3326,2980, 2936, 1744, 1665, 1606, 1560
nmr($CDCl_3$) δ	:	1.31 (m, 12H, Ala CH_3 + Boc CH_3), 1.86 (ss, 6H, AEH CH_3 × 2), 2.28 (s, 3H, CH_3), 3.0 (d, 2H, $C^{\beta}H_2$), 3.5-4.17 (m, 8H, $COOCH_3$ + -CH_2-CH_2- + Ala $C^{\alpha}H$), 4.56-5.16 (m, 3H, Tyr $C^{\alpha}H$ + enolic CH + Ala NH), 6.56 (d, 1H, Tyr NH), 6.72 (d, 1H, Tyr C-5 H), 6.94 (d, 1H, Tyr C-6 H), 7.19 (s, 1H, Tyr C-2 H), 10.81 (s, 1H, enolic OH), 15.15 (s, 1H, phenolic OH)
ms (m/z)	:	533 $(MH)^+$

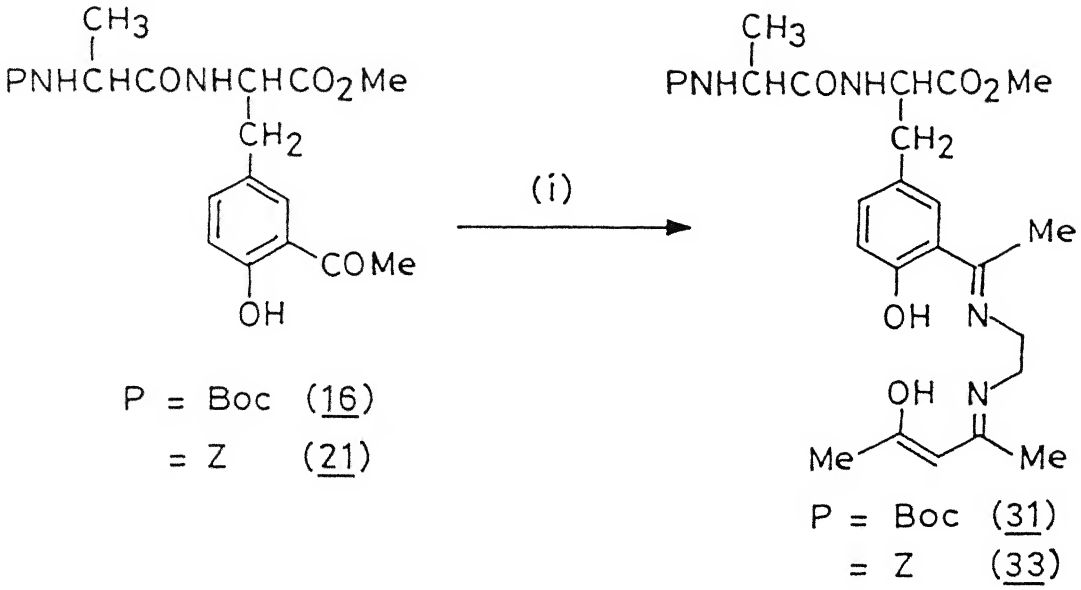
(32)

yield	:	72%
mp	:	164°C
ir(KBr)ν_{max} cm^{-1}	:	3276, 2928, 1743, 1668, 1635, 1537, 1485
nmr($CDCl_3$) δ	:	1.34 (m, 12H, Ala CH_3 + Boc CH_3), 1.93 (s, 6H, AEH CH_3), 2.62 (s, 3H, CH_3), 3.12 (d, 2H, Tyr $C^{\beta}H_2$), 3.43-4.25 (m, 10H, $COOCH_3$ + -CH_2-CH_2- + Ala $C^{\alpha}H$ + Ser $C^{\beta}H_2$), 4.68 (m, 2H, Tyr $C^{\alpha}H$ + Ser $C^{\alpha}H$), 5.0 (s, 1H, enolic CH), 5.31 (d, 1H, Ala NH), 6.78-7.78 (m, 5H, Tyr NH + Ser NH + aromatic) , 10.93 (s, 1H, enolic OH), 12.15 (s, 1H, phenolic OH)
ms (m/z)	:	620 $(MH)^+$

(33)

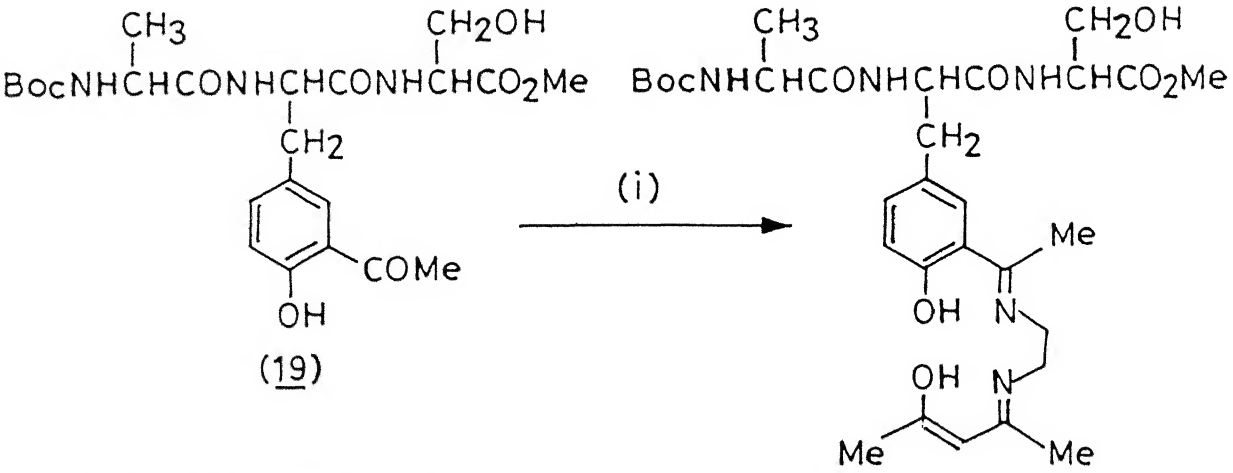
yield	:	71%
mp	:	215°C
ir(KBr)ν_{max} cm^{-1}	:	3310, 3060, 2956, 1740, 1682, 1649, 1610, 1525
nmr($CDCl_3$-DMSO-d_6) δ	:	1.31 (m, 3H, Ala CH_3), 1.97 (ss, 6H, AEH CH_3 × 2), 2.56 (s, 3H, CH_3), 3.09 (d, 2H, Tyr $C^{\beta}H_2$), 3.72 (m, 7H, $COOCH_3$ + -CH_2-CH_2-), 4.19 (m, 1H, Ala $C^{\alpha}H$), 4.88 (q, 1H, Tyr $C^{\alpha}H$), 5.0 (s, 1H, enolic CH), 5.09 (s, 2H, Z CH_2), 5.56 (d, 1H, Ala NH), 6.75-7.63 (m, 9H, Tyr NH + aromatic) , 10.94 (s, 1H, enolic OH)

CHART C.I.10



P = Boc (16)
= Z (21)

P = Boc (31)
= Z (33)



(19)

(32)

(i) AEH (11) / MeOH, 2h

The NMR spectra of the compounds were in complete agreement with the structures proposed and a noteworthy feature is the presence of the phenolic proton at $\sim$ 15 ppm and the AEH hydroxyl proton at $\sim$ 11 ppm. The mass spectra of the compounds showed strong peaks corresponding to $(MH)^+$. Interestingly, they also exhibited small peak that matched with products that could be derived from the treatment of the precursors with ethylenediamine. On the basis of careful examination of all the data it is concluded that the latter peak arises as an artifact in the spectrometer involving intervention of the matrix.

Thus, the result presented in CHART C.I.10 clearly shows that Tyr(3-Ac) in a peptide environment clearly forms the expected templates with AEH.

An interesting aspect relevant in the domain of protein restructuring would be the linking of proximate tyrosine residues in proteins to afford templates that have metal uptake potential. Work thus far reported would show that this could be readily achieved in a peptide environment by treatment of Tyr(3-Ac) side chain with ethylenediamine. This, as shown in CHART C.I.11, has been readily experimentally illustrated by treatment of ZAlaTyr(3-Ac)OMe (21), ZSerTyr(3-Ac)OMe (25) and ZAlaTyr(3-Ac)SerOMe (23) with ethylenediamine, in MeOH at room temperature for 12 h to afford, respectively, templates (34), (36) and (35) in excellent yields (CHART C.I.11).

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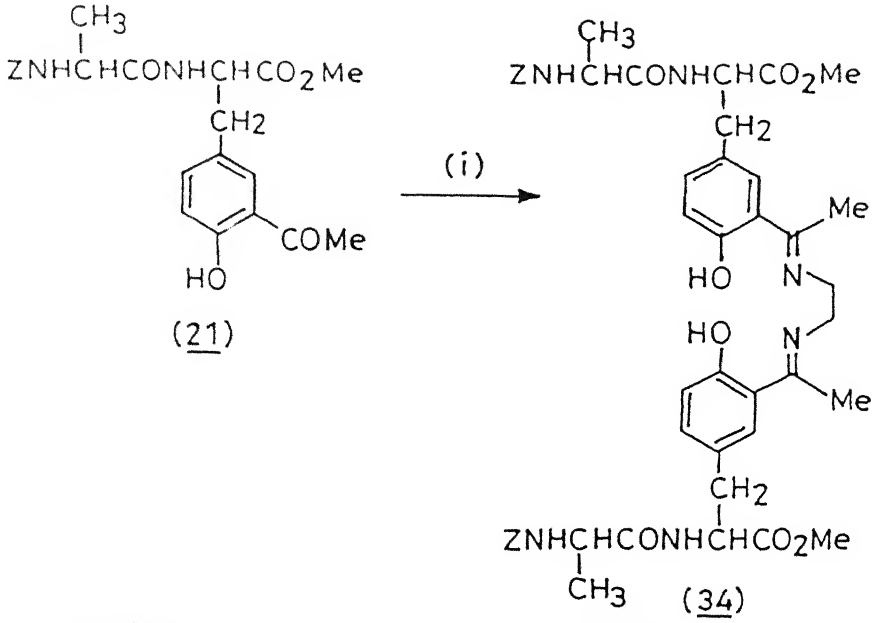
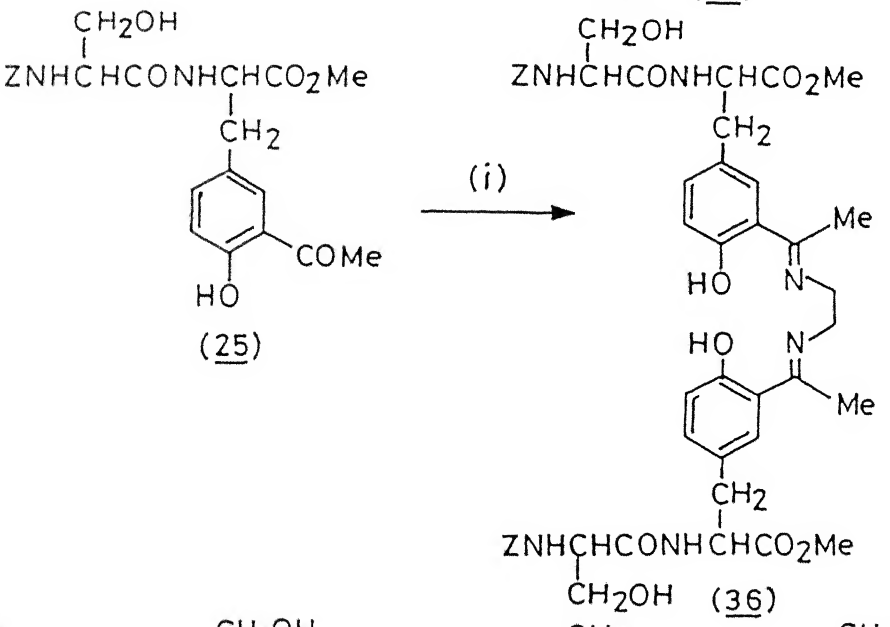
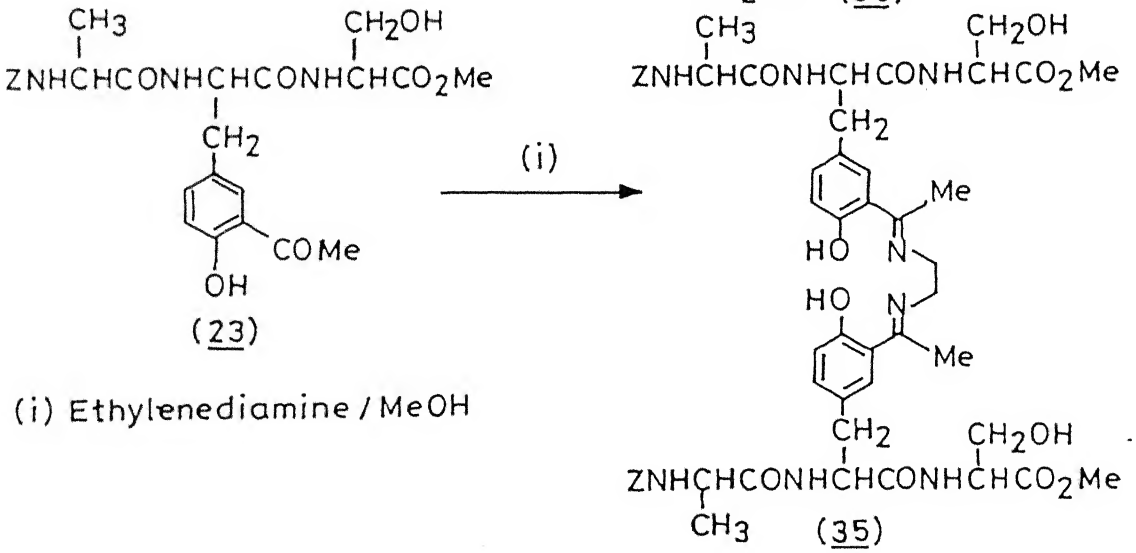
(34)

yield	:	88%
mp	:	213-215°C
ir(KBr)ν_{max} cm^{-1}	:	3302, 1717, 1647, 1608, 1522
ms (m/z)	:	909 $(MH)^+$

CHART C.I.11







(i) Ethylenediamine / MeOH

(35)

yield	:	89%
mp	:	249-251°C
ir(KBr)ν_{max} cm^{-1}	:	3286, 1740, 1683, 1628, 1528
ms (m/z)	:	1083 $(MH)^+$

(36)

yield	:	90%
mp	:	155-158°C
ir(KBr)ν_{max} cm^{-1}	:	3295, 2927, 1723, 1680, 1645, 1606, 1524
ms (m/z)	:	941 $(MH)^+$

As stated previously oximes, AEH and ethylenediamine adducts derived from Tyr(3-Ac) readily form metal templates. This aspect is illustrated with the simple substrate BzTyr(3-Oximinoacetyl)OMe (7) by treatment with $Cu(OAc)_2$, $Ni(OAc)_2$, and $Co(OAc)_2$, in MeOH for 0.5 h leading to the clean precipitation of the corresponding templates (37), (38) and (39) (CHART C.I.12). The EPR spectrum of (37) taken at room temperature showed the characteristic four line pattern and the expected five line hyperfine splitting

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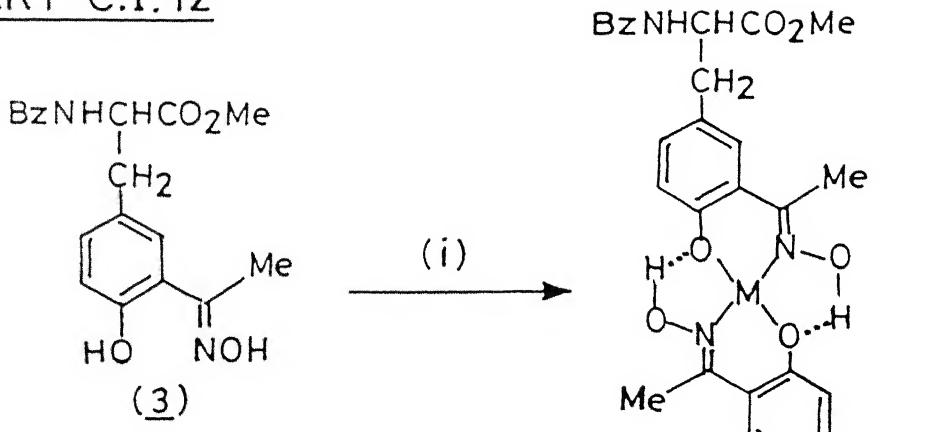
(37)

yield	:	78%
mp	:	253°C
ir(KBr)ν_{max} cm^{-1}	:	3289, 2951, 1741, 1637, 1780, 1531
epr($CHCl_3$, rt)	:	$A_{\|\|} = 95$, $g_{\|\|} = 2.112$, $g_{\perp} = 2.013$
ms (m/z)	:	774 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm	:	259, 345 647

(38)

yield	:	83%
mp	:	265°C
ir(KBr)ν_{max} cm^{-1}	:	3287, 3027, 2923, 1740, 1636

CHART C.I.12



M	(X)
Cu(II)	(37)
Ni(II)	(38)
Co(II)	(39)

(i) $M(OAc)_2$ – MeOH, 0.5 h

nmr($CDCl_3$) δ	:	2.31 (s, 6H, $CH_3 \times 2$), 3.15 (d, 4H, $C^{\beta}H_2 \times 2$), 3.75 (s, 6H, $COOCH_3 \times 2$), 5.0 (q, 2H, $C^{\alpha}H \times 2$), 6.56 (d, 2H, NH × 2), 6.69-7.93 (m, 16H, aromatic), 10.87 (s, 2H, N-OH × 2)
ms (m/z)	:	769 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm	:	259, 301, 382 602

(39)

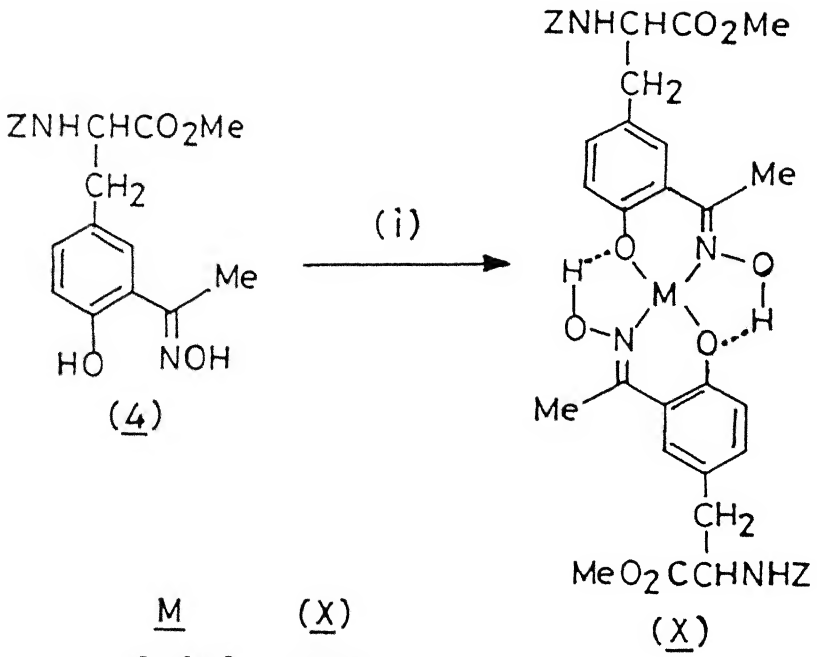
yield	:	85%
mp	:	151°C (dec.)
ir(KBr)ν_{max} cm^{-1}	:	3314, 1717, 1646, 1540, 1492
ms (m/z)	:	770 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm	:	255, 311 650

of the fourth line indicating metal co-ordination is through two nitrogens and two oxygens. This profile is characteristic of square planar copper complexes. The nature of co-ordination in (37) places the oximino hydroxyl group anti to the metal center and this fact could be taken advantage of to attach additional ligands to this function. The FAB mass spectrum of (37) also supported the assigned structure. The NMR spectrum of (38) was very similar to that of parent template excepting for the non appearance of the peak expected of the phenolic proton around 12 ppm. In the case of (38) the FAB mass showed the parent ion at 769 as the base peak.

Similarly, the structural assignment for the cobalt template (39) is supported by spectral and analytical data. The expected absorbance corresponding to the d-d transition in these compounds were seen in the UV-VIS spectrum around 600 nm.

ZTyr(3-Oximinoacetyl)OMe (8) afforded Cu(II) and Ni(II) complexes (40) and (41) on treatment with the corresponding metal acetate in MeOH at room temperature for 0.5 h (CHART C.I.13).

CHART C.I.13



M	(X)
Cu(II)	(40)
Ni(II)	(41)

(i) $M(OAc)_2$ – MeOH, 0.5 h

(40)

yield	:	73%
mp	:	208°C
ir(KBr)ν_{max} cm^{-1}	:	3318, 2950, 1740, 1690, 1636, 1479
epr($CHCl_3$, rt)	:	$A_{\|\|} = 100$, $g_{\|\|} = 2.115$, $g_{\perp} = 2.005$
ms (m/z)	:	896, 835 $(MH)^+$
uv-vis (DMSO)λ_{max} nm	:	259, 419, 646

(41)

yield	:	75%
mp	:	213-215°C
ir(KBr)ν_{max} cm^{-1}	:	3324, 2921, 1737, 1688, 1632, 1528
ms (m/z)	:	829 $(MH)^+$
uv-vis (DMSO)λ_{max} nm	:	260, 305, 392, 601

Similarly, AcTyr(3-Oximinoacetyl)OMe (9) afforded the copper template (42) and the nickel template (43) (CHART C.I.14).

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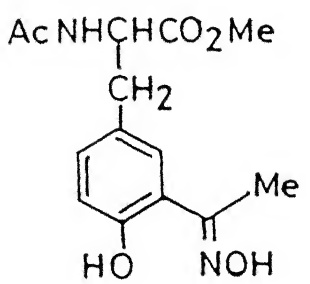
(42)

yield	:	76%
mp	:	270-272°C
ir(KBr)ν_{max} cm^{-1}	:	3304, 2924, 1741, 1654, 1545, 1479
epr($CHCl_3$, rt)	:	$A_{\|\|} = 100$, $g_{\|\|} = 2.114$, $g_{\perp} = 2.013$
ms (m/z)	:	651 $(MH)^+$
uv-vis (DMSO)λ_{max} nm	:	269, 390, 584

(43)

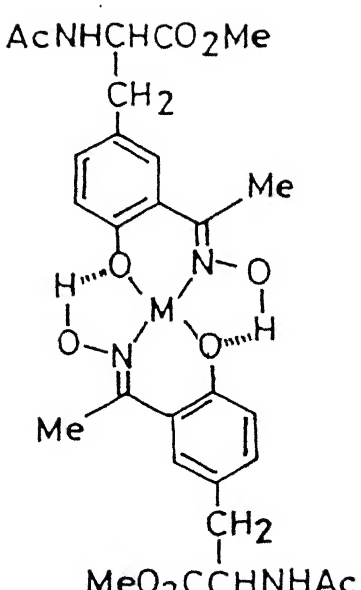
yield	:	81%
mp	:	292-295°C
ir(KBr)ν_{max} cm^{-1}	:	3448, 3292, 1736, 1652, 1552, 1507
ms (m/z)	:	645 $(MH)^+$
uv-vis	:	262, 304, 366, 468(sh), 599

CHART C.I.14



(6)

(i) →



(X)

M	(X)
Cu(II)	(42)
Ni(II)	(43)

(i) $M(OAc)_2$–MeOH, 0.5 h

The metal templates (37) to (43) described above necessitated the involvement of two Tyr units. In this context the ready metal complexation of the Tyr-AEH composite is significant since such a structural combination can be readily arrived from Tyr(3-Ac) and AEH units.

Thus the reaction of BzTyr(3-Ac)OMe - AEH adduct (12) readily afforded the copper template (44) and the nickel and cobalt analogs (45) and (46), respectively, on treatment with corresponding metal(II) acetate in MeOH for 1.5 h (CHART C.I.15).

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(44)

yield	:	74%
mp	:	115-118°C
ir(KBr)ν_{max} cm^{-1}	:	3447, 3061, 2949, 1735, 1641, 1592, 1514
epr(MeOH, rt)	:	$A_{\|\|} = 90$, $g_{\|\|} = 2.111$, $g_{\perp} = 2.016$
(MeOH, -196°C)	:	$A_{\|\|} = 185$, $g_1 = 2.359$, $g_2 = 2.087$, $g_3 = 1.999$
ms (m/z)	:	527 $(MH)^+$
uv-vis $(CHCl_3)\lambda_{max}$ nm	:	246, 328, 387, 546

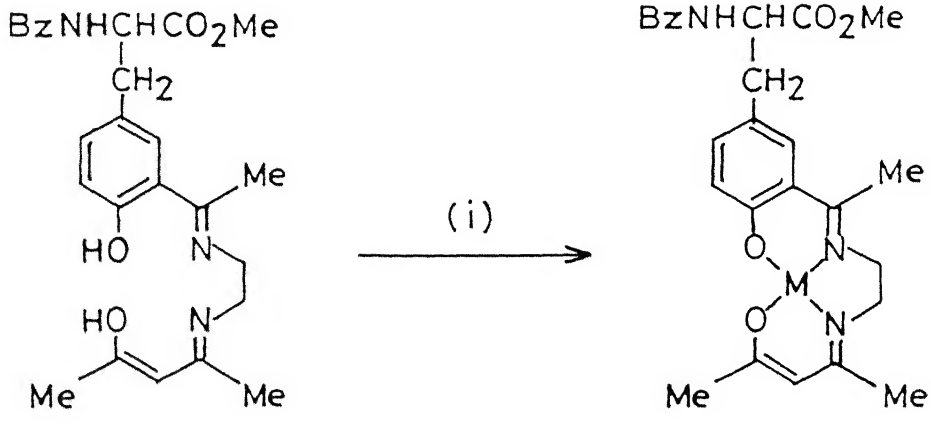
(45)

yield	:	80%
mp	:	130°C
ir(KBr)ν_{max} cm^{-1}	:	3421, 2950, 1741, 1638, 1615, 1578, 1517
nmr($CDCl_3$) δ	:	1.88 (d, 6H, AEH CH_3), 2.19 (s, 3H, CH_3), 2.97-3.53 (m, 6H, $-CH_2-CH_2-$ + $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.97 (s, 1H, =CH), 5.0 (q, 2H, $C^{\alpha}H$), 6.63 (d, 1H, NH), 6.81-7.88 (m, 8H, aromatic)
ms (m/z)	:	521 $(MH)^+$
uv-vis $(CHCl_3)\lambda_{max}$ nm	:	250, 333, 412 562

(46)

yield	:	71%
mp	:	168° C

CHART C.I.15



(12) (X)

M	(X)
Cu(II)	(44)
Ni(II)	(45)
Co(II)	(46)

(i) $M(OAc)_2$ – MeOH, 0.5 h

ir(KBr)ν_{max} cm^{-1}	:	3398, 2925, 1741, 1616, 1560
ms (m/z)	:	522 $(MH)^+$
uv-vis $(CHCl_3)\lambda_{max}$ nm	:	241, 389(sh), 761

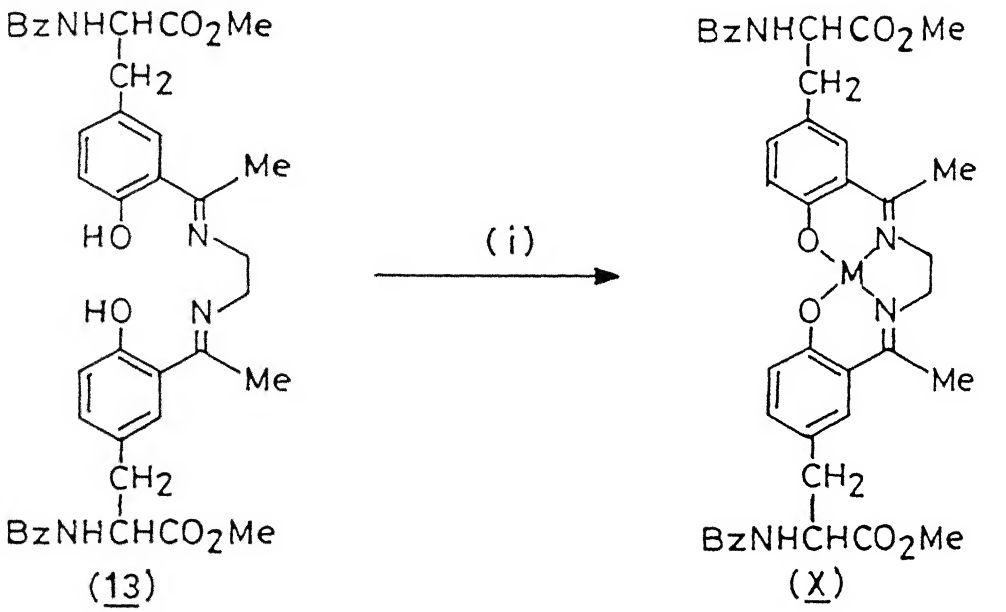
The EPR profile of (44) was similar to that of (37) which was as expected. In the FAB mass spectrum, the base peak was at 527 corresponding to the parent ion. The NMR spectrum of the nickel template (45) had a profile similar to that of its precursor (12) excepting for a significant upfield shift of the AEH protons and the absence of peaks corresponding to the hydroxyl groups of the phenol and the AEH. Here again the FAB mass spectrum exhibited the peak at 521 corresponding to the parent ion. The cobalt template (46) had a base peak at 522 in the FAB mass spectrum corresponding to the parent ion.

The importance of linking of pairs of Tyr(3-Ac) units in a peptide environment with ethylenediamine to afford sites for metal complexation has been stated earlier. This has been experimentally demonstrated with the BzTyr(3-Ac)OMe - ethylenediamine adduct (13), which afforded in excellent yields Cu(II) (47), Ni(II) (48) and Co(II) (49) complexes on treatment of their corresponding metal acetates in MeOH-MeCN at room temperature for 1 h (CHART C.I.16).

(47)

yield	:	78 %
mp	:	148-149°C
ir(KBr)ν_{max} cm^{-1}	:	3378, 2951, 1740, 1646, 1615, 1586, 1530
epr($CHCl_3$, rt)	:	$A_{\|\|} = 100$, $g_{\|\|} = 2.112$, $g_{\perp} = 2.011$
($CHCl_3$, -196°C)	:	$A_{\|\|} = 205$, $g_1 = 2.193$, $g_2 = 2.053$, $g_3 = 1.980$
ms (m/z)	:	769 $(MH)^+$
uv-vis $(CHCl_3)\lambda_{max}$ nm	:	245, 371, 550

CHART C.I.16



(13) (X)

M	(X)
Cu(II)	(47)
Ni(II)	(48)
Co(II)	(49)

(i) $M(OAc)_2$ – MeOH / MeCN, 1h

(48)

yield	:	80 %
mp	:	206-208 °C
ir(KBr)ν_{max} cm^{-1}	:	3431, 2952, 1734, 1646, 1615, 1578, 1531
nmr($CDCl_3$) δ	:	2.0 (s, 6H, CH_3 × 2), 3.13 (d, 4H, $C^{\beta}H_2$ × 2), 3.47 (m, 4H, -CH_2-CH_2), 3.66 (s, 6H, $COOCH_3$ × 2), 5.03 (q, 2H, $C^{\alpha}H$ × 2), 6.66-7.94 (m, 18H, NH × 2 + aromatic)
ms (m/z)	:	763 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm	:	260, 339, 415 560

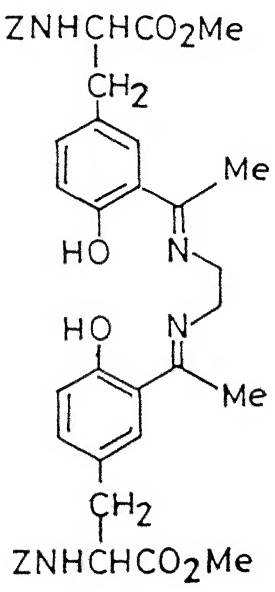
(49)

yield	:	72%.
mp	:	165°C
ir(KBr)ν_{max} cm^{-1}	:	3352, 2926, 1738, 1648, 1615, 1540, 1484
ms (m/z)	:	764 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm	:	246, 365, 635

The EPR profile of complex (47) taken in methanol and chloroform were similar to those reported previously. In addition compound (47) also afforded clean EPR spectrum at liquid nitrogen temperature (LNT), enabling the estimation of $A_{\|}$, $g_{\|}$, and $g_{\perp}$ values which as expected supported square planar configuration. The appearance of peak at 769 in the FAB mass spectrum as the base peak was quite remarkable attesting to the very stable nature of the metal complexes. The surprising feature of the NMR of (48) in $CDCl_3$ was the presence of a single peak corresponding to 4 protons which was determined as arising from water co-ordination by exchange studies. As expected, compound (48) did not show the presence of phenolic proton in the NMR. Here also the strong base peak corresponding to the parent ion was seen in the FAB mass spectrum.

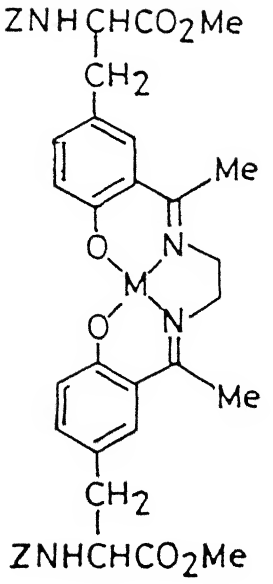
A similar set of Tyr(3-Ac) - ethylenediamine link Cu(II) (50), Ni(II) (51) and Co(II) (52) complexes were prepared by treatment of the metal acetates with ethylenediamine link of ZTyr(3-Ac)OMe (14) in MeOH (CHART C.I.17).

CHART C.I.17



(14)

(i)



(X)

(M)	(X)
Cu (II)	(50)
Ni (II)	(51)
Co (II)	(52)

(i) $M(OAc)_2$–MeOH, 1h

(50)

yield	:	74%
mp	:	115°C
ir(KBr)ν_{max} cm^{-1}	:	3329, 2950, 1720, 1614, 1587, 1529
epr($CHCl_3$, rt)	:	$A_{\|\|} = 95$, $g_{\|\|} = 2.111$, $g_{\perp} = 2.013$
($CHCl_3$, -196°C)	:	$A_{\|\|} = 215$, $g_1 = 2.1909$, $g_2 = 2.0424$, $g_3 = 1.9783$
uv-vis (DMSO)λ_{max}nm	:	272, 373, 559

(51)

yield	:	78%
mp	:	190-195°C
ir(KBr)ν_{max} cm^{-1}	:	3346, 2923, 1718, 1613, 1578, 1529
nmr($CDCl_3$) δ	:	1.75 (s, 6H, CH_3 x 2), 2.88 (br, 4H, $C^{\beta}H_2$ x 2), 3.50 (s, 4H, -CH_2CH_2-), 3.72 (s, 6H, $COOCH_3$ x 2), 4.47 (br, 2H, $C^{\alpha}H$ x 2), 5.06 (s, 4H, Z CH_2 x 2), 5.47 (brd, 2H, NH x 2), 6.91 (m, 6H, Tyr aromatic), 7.34 (s, 10H, Z aromatic)
uv-vis (DMSO)λ_{max}nm	:	263, 337, 410, 557

(52)

yield	:	75%
mp	:	157-162°C
ir(KBr)ν_{max} cm^{-1}	:	3413, 2928, 1720, 1616, 1561, 1481
uv-vis (DMSO)λ_{max} nm	:	263, 375, 554

The EPR spectrum of (50) recorded at room temperature and at LNT showed the expected nearly square planar profile. The NMR spectrum of the Ni complex (51) surprisingly exhibited peaks that were broad in contrast to the corresponding benzoyl derivative (48). In addition the four ethylenediamine protons were upshifted and were non equivalent. Otherwise the NMR spectrum was quite clean as expected.

The work presented thus far has clearly shown that metal uptake systems can be very readily constructed from Tyr(3-Ac)OMe either by oximination or by Schiff base

formation with AEH or by linking with ethylenediamine. Logically the next phase would be to experimentally demonstrate that the Tyr(3-Ac) side chain in a peptide would also undergo similar transformations. This aspect has been successfully demonstrated experimentally.

BocAlaTyr(3-Oximinoacetyl)OMe (26) readily formed the expected Cu(II) (53), Ni(II) (54) and Co(II) (55) complexes on treatment with the corresponding metal acetates in MeOH at room temperature for 0.5 h (CHART C.I.18).

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(53)

yield	:	67%
mp	:	226°C
ir(KBr)ν_{max} cm^{-1}	:	3387, 3329, 2977, 1741, 1666, 1520
epr($CHCl_3$, rt)	:	$A_{\|\|} = 100$, $g_{\|\|} = 2.116$, $g_{\perp} = 2.013$
uv-vis ($CHCl_3$)λ_{max} nm	:	254, 345, 648

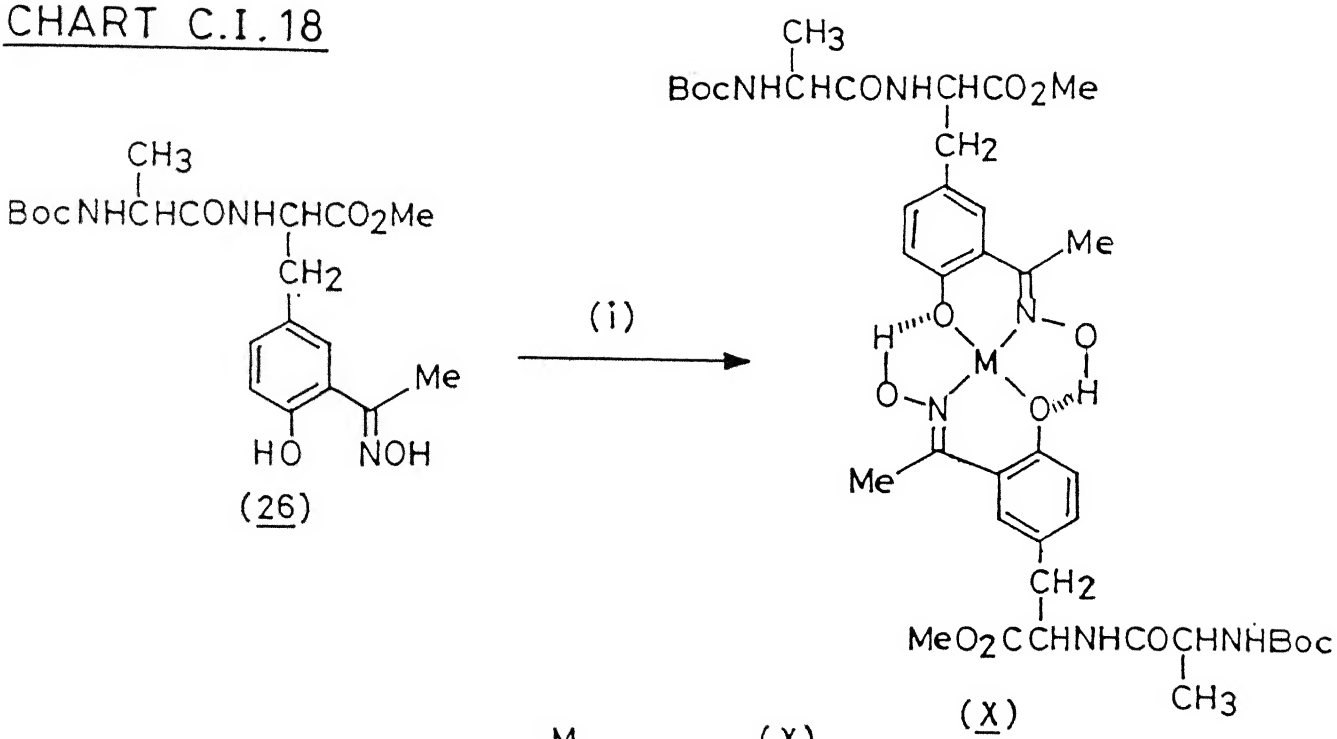
(54)

yield	:	69%
mp	:	216-217°C
ir(KBr)ν_{max} cm^{-1}	:	3327, 2977, 1740, 1667, 1613, 1522
nmr($CDCl_3$) δ	:	1.34 (m, 24H, Boc CH_3 x 6 + Ala CH_3 x 2), 2.38 (s, 6H, CH_3 x 2), 3.0 (d, 4H, Tyr $C^{\beta}H_2$ x 2), 3.69 (s, 6H, $COOCH_3$ x 2), 4.06 (m, 2H, Ala $C^{\alpha}H$ x 2), 4.72 (m, 4H, Tyr $C^{\alpha}H$ x 2 + Ala NH x 2), 6.47 (d, 2H, Tyr NH x 2), 6.53-7.31 (m, 6H, aromatic), 10.84 (s, 2H, NOH x 2)
ms (m/z)	:	902 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm	:	256, 301, 381, 602

(55)

yield	:	72%
mp	:	157°C (dec.)

CHART C.I.18



M	(X)
Cu(II)	(53)
Ni(II)	(54)
Co(II)	(55)

(i) $M(OAc)_2$ – MeOH, 0.5 h

ir(KBr)ν_{max} cm^{-1}	:	3341, 2929, 1742, 1691, 1670, 1539, 1510
ms (m/z)	:	903$(M)^+$
uv-vis $(CHCl_3)\lambda_{max}$ nm	:	252, 300(sh), 659(sh)

The EPR spectrum of (53) showed co-ordination with pairs of nitrogen in accordance with the square planar configuration shown in CHART C.I.18.

The NMR spectrum of (54) was very similar to its metal precursor (26) excepting the fact the Boc-NH proton was significantly shifted upfield and the absence of peak corresponding to the phenolic hydroxyl group. Compound (55) exhibited strong peak in the FAB mass spectrum at 903 corresponding to the parent ion.

In BocAlaTyr(3-Oximinoacetyl)SerOMe (27) the Tyr(3-Ac) side chain is truly in a non terminal location and the ready formation of Cu(II) (56), Ni(II) (57) and Co(II) (58) complexes by treatment of the corresponding metal acetates in MeOH at room temperature for 0.5 h from (27) is therefore significant (CHART C.I.19).

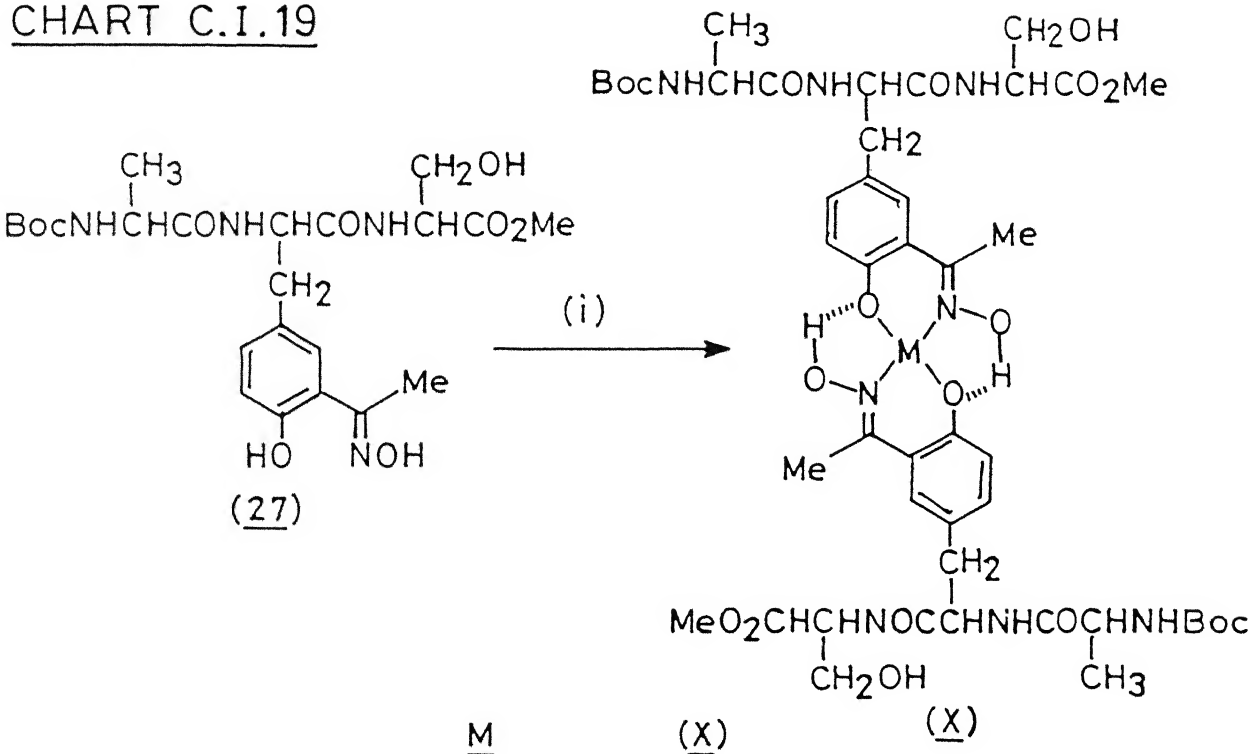
(56)

yield	:	84%
mp	:	255°C (dec.)
ir(KBr)ν_{max} cm^{-1}	:	3315, 2977, 2929, 1744, 1652, 1510
epr(DMSO, -196°C)	:	$A_{\|\|} = 195$, $g_1 = 2.236$, $g_2 = 2.085$, $g_3 = 2.016$
uv-vis (DMSO)λ_{max} nm	:	263, 342, 631

(57)

yield	:	72%
mp	:	252°C
ir(KBr)ν_{max} cm^{-1}	:	3419, 2925, 2854, 1741, 1652, 1508
ms (m/z)	:	1076 $(M)^+$

CHART C.I.19



M	(X)
Cu(II)	(56)
Ni(II)	(57)
Co(II)	(58)

(i) $M(OAc)_2$ – MeOH, 0.5 h

(58)

yield	:	68%
mp	:	>300°C
ir(KBr)ν_{max} cm^{-1}	:	3416, 2976, 2923, 1717, 1684, 1654, 1558, 1540
ms (m/z)	:	1077 $(M)^+$

The EPR spectrum of the Cu(II) complex (56) derived from BocAlaTyr(3-Oximinoacetyl)SerOMe (27) reflected the consequences of increasing steric bulk around the template. Thus, unlike the case of templates derived either from N,C- protected Tyr(3-Oximinoacetyl) unit or dipeptides having this moiety, where, clean square planar profile with expected hyperfine splitting was observed, the EPR profile of (56) showed considerable rhombic distortion. This property was found in similar cases as well *vide infra*. The presence of peak at 1077 in the FAB mass spectrum of (58) as the base peak corresponding to the parent ion again highlights the stability of this class of metal complexes.

ZAlaTyr(3-Oximinoacetyl)OMe (28), similarly, afforded Cu(II) (59) and Ni(II) (60) complexes (CHART C.I.20).

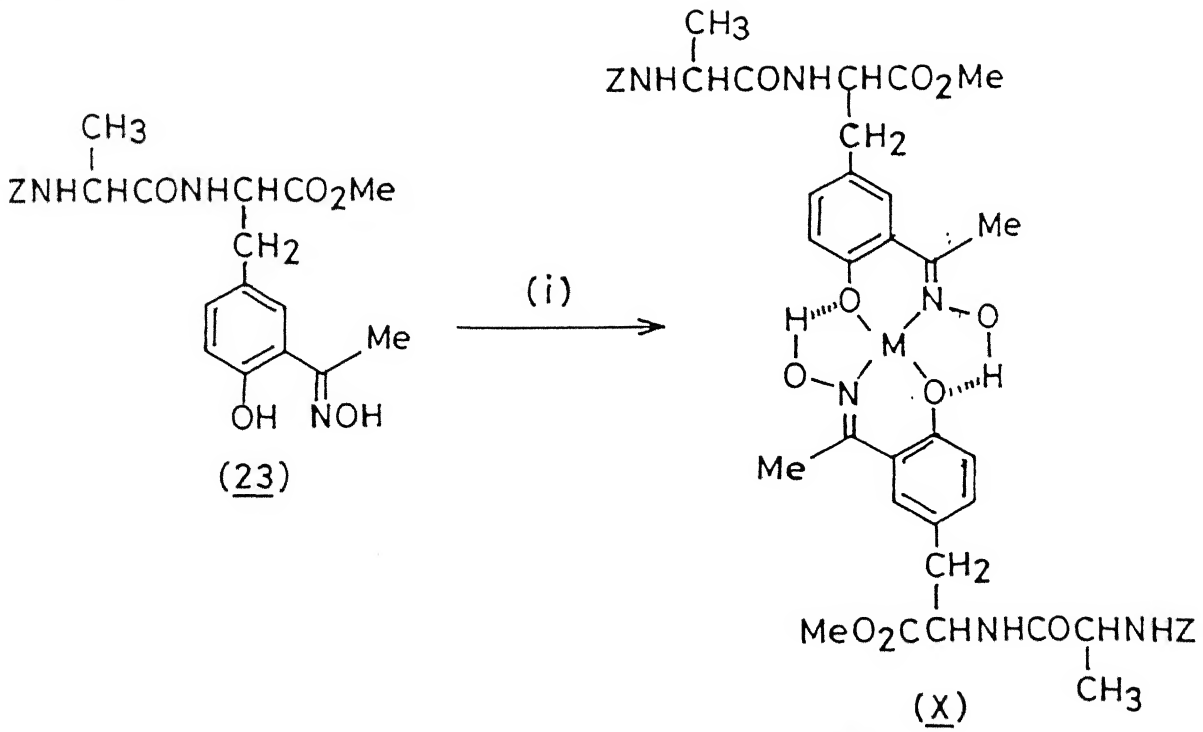
(59)

yield	:	80%
mp	:	225-228°C
ir(KBr)ν_{max} cm^{-1}	:	3298, 2942, 1722, 1680, 1635, 1522
epr(DMF, -196°C)	:	$A_{\|\|} = 195$, $g_1 = 2.079$, $g_2 = 2.053$, $g_3 = 2.001$
uv-vis (DMSO)λ_{max} nm	:	267, 334, 386, 594

(60)

yield	:	78%
mp	:	230-233°C
ir(KBr)ν_{max} cm^{-1}	:	3302, 3033, 2927, 1740, 1689, 1651, 1540
ms (m/z)	:	972 $(MH)^+$
uv-vis (DMSO)λ_{max} nm	:	266, 300, 373, 598

CHART C.I.20



M	(X)
Cu(II)	(59)
Ni(II)	(60)

(i) $M(OAc)_2$ – MeOH , 0.5h

Similarly, ZAlaTyr(3-Oximinoacetyl)SerOMe (39), wherein, the Tyr(3-Oximinoacetyl) unit is placed in a non terminal location, readily afforded the copper complex (61) (CHART C.I.21).

---

(61)

yield	:	64%
mp	:	245-247°C
ir(KBr)ν_{max} cm^{-1}	:	3288, 3064, 2928, 1744, 1694, 1646, 1541
epr(DMSO, -196°C)	:	$A_{\|\|} = 195$, $g_1 = 2.220$, $g_2 = 2.055$, $g_3 = 2.004$
uv-vis (DMSO)λ_{max} nm	:	260, 358, 423(sh), 654

Expected Cu(II) (62) and Ni(II) (63) complexes were obtained from ZSerTyr(3-Oximinoacetyl)OMe (30) (CHART C.I.22).

---

(62)

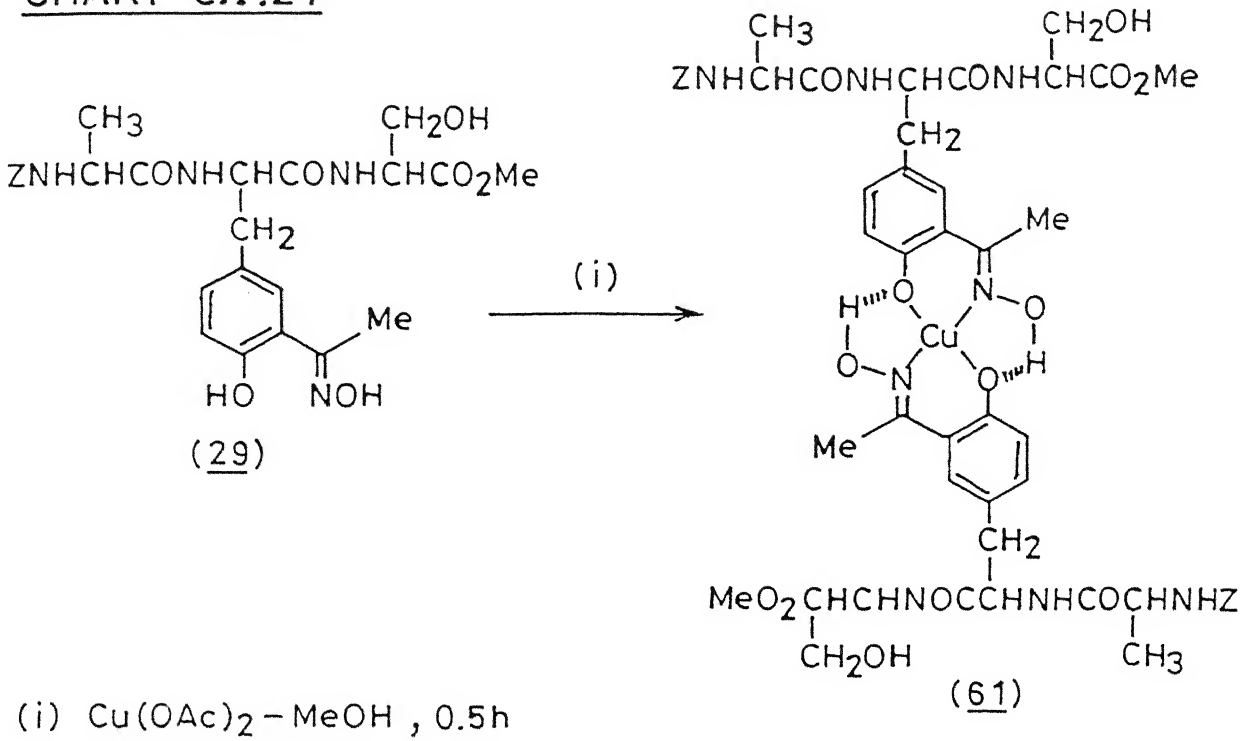
yield	:	82%
mp	:	198-202°C
ir(KBr)ν_{max} cm^{-1}	:	3302, 3068, 2947, 1719, 1640, 1605, 1530
epr(DMSO, -196°C)	:	$A_{\|\|} = 190$, $g_1 = 2.225$, $g_2 = 2.056$, $g_3 = 2.004$
uv-vis (DMSO)λ_{max} nm	:	263, 344, 380, 629

(63)

yield	:	73%
mp	:	228-230°C
ir(KBr)ν_{max} cm^{-1}	:	3302, 2926, 1735, 1695, 1652, 1541
ms (m/z)	:	1004 $(MH)^+$
uv-vis (DMSO)λ_{max} nm	:	306, 374, 599

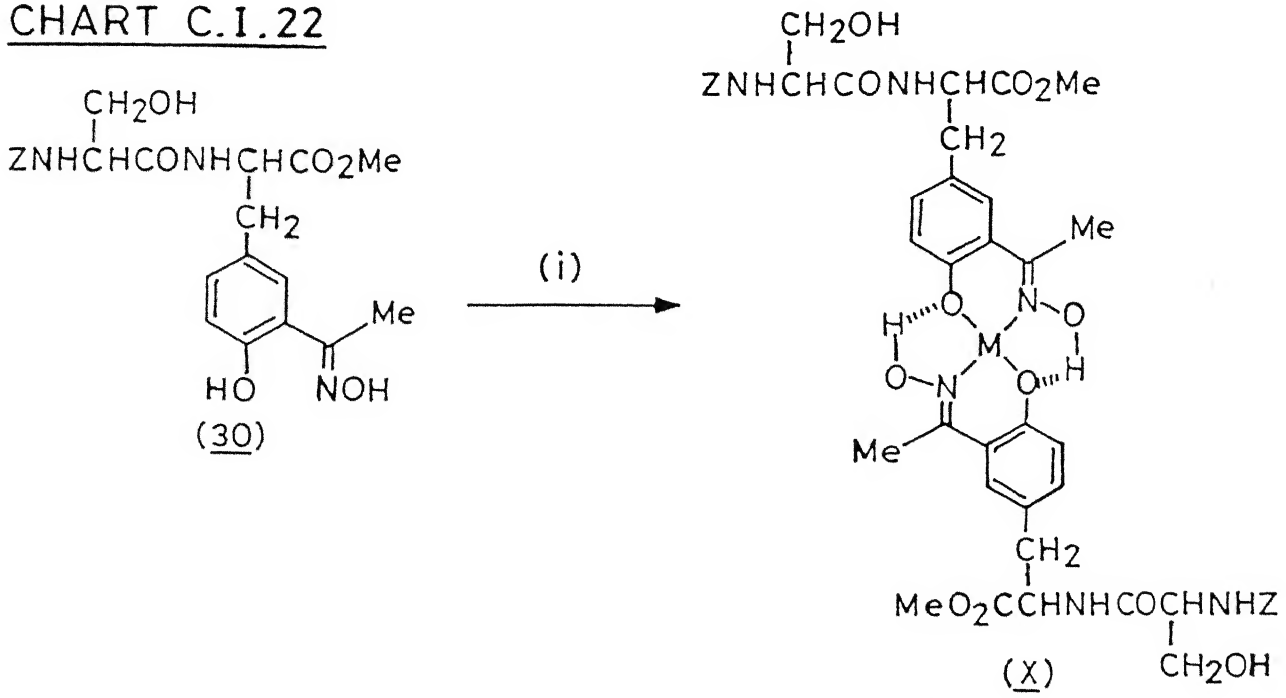
The real practical utility of the present endeavours would be the demonstration of methodologies, wherein, the Tyr(3-Ac) residue in a peptide environment, can be

CHART C.I.21



(i) $Cu(OAc)_2$ – MeOH , 0.5h

CHART C.I.22



M	(X)
Cu(II)	(62)
Ni(II)	(63)

(i) $M(OAc)_2$ – MeOH, 0.5h

been experimentally demonstrated.

Reaction of BocAlaTyr(3-Ac)OMe-AEH composite (31) on treatment with $Cu(OAc)_2$ in MeOH at room temperature for 1 h readily afforded the expected Cu(II) template (64) in 68% yields (CHART C.I.23).

---

(64)

yield	:	68%
mp	:	125-130°C
ir(KBr)ν_{max} cm^{-1}	:	3300, 1730, 1650, 1580, 1500
epr(MeOH, rt)	:	$A_{\|\|} = 90$, $g_{\|\|} = 2.110$, $g_{\perp} = 2.017$
(MeOH, -196°C)	:	$A_{\|\|} = 195$, $g_1 = 2.207$, $g_2 = 2.042$, $g_3 = 1.990$
ms (m/z)	:	594 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm	:	274, 313, 382(sh), 547, 650(sh)

The EPR profile of (64) both at room temperature and at LNT was excellent and the relevant parameters (*vide supra*) show that the complex is square planar. The FAB mass spectrum exhibited peak at 594 corresponding to the parent ion.

BocAlaTyr(3-Ac)SerOMe-AEH template (32), wherein, the Tyr(3-Ac) unit is truly in a peptide environment, afforded the desired Cu(II) template (65) on treatment with $Cu(OAc)_2$ in MeOH at room temperature for 1 h (CHART C.I.24).

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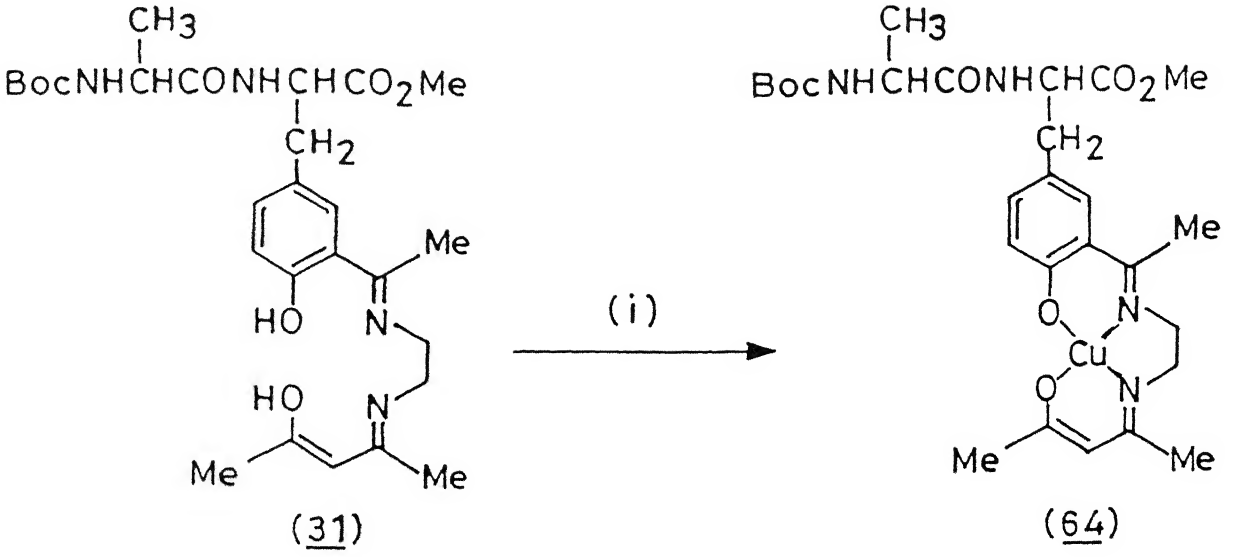
(65)

yield	:	73%
mp	:	142-150°C
ir(KBr)ν_{max} cm^{-1}	:	3410, 3290, 1730, 1632, 1580, 1503
epr($CHCl_3$, rt)	:	$A_{\|\|} = 90$, $g_{\|\|} = 2.109$, $g_{\perp} = 2.009$
($CHCl_3$, -196°C)	:	$A_{\|\|} = 205$, $g_1 = 2.1858$, $g_2 = 2.0346$, $g_3 = 2.0346$

Compound (65) exhibited excellent EPR profile both at room temperature and at LNT. The appropriate parameters thus derived (*vide supra*) clearly show the compound

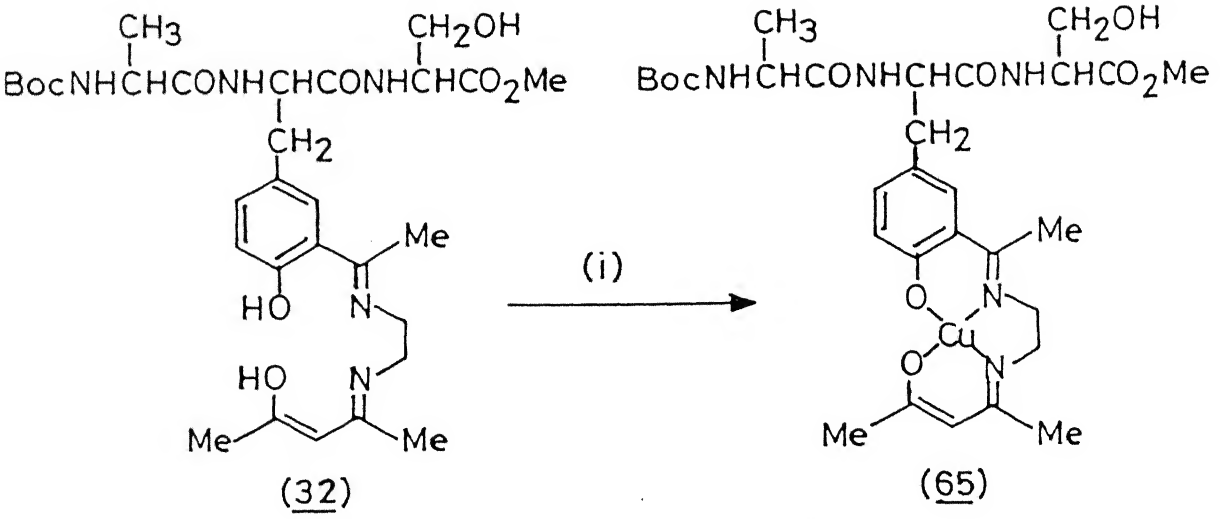
CHART C.I.23



(31) (64)

(i) $Cu(OAc)_2$ – MeOH , 1h

CHART C.I.24



(32) (65)

(i) $Cu(OAc)_2$ – MeOH, 1h

is square planar. Interestingly, the base peak in the FAB mass spectrum appeared at 705 which strongly suggests that on electron impact the Cu(II) → Cu(I) change takes place readily and the resulting negative charge species complexes with sodium ion. The resulting (bi-metallic cluster + H)$^+$ would account for the peak at 705.

The feasibility of bridging two Tyr(3-Ac) units with ethylenediamine to form templates that can readily accept metal ions has been demonstrated in CHART C.I.16 and in CHART C.I.17. That this can be achieved in a peptide environment has also been demonstrated. Thus, ZAlaTyr(3-Ac)OMe - ethylenediamine composite (34) readily afforded the Cu(II) (66) and Ni(II) (67) templates on treatment with their corresponding metal acetates in MeOH at room temperature for 1 h (CHART C.I.25).

---

(66)

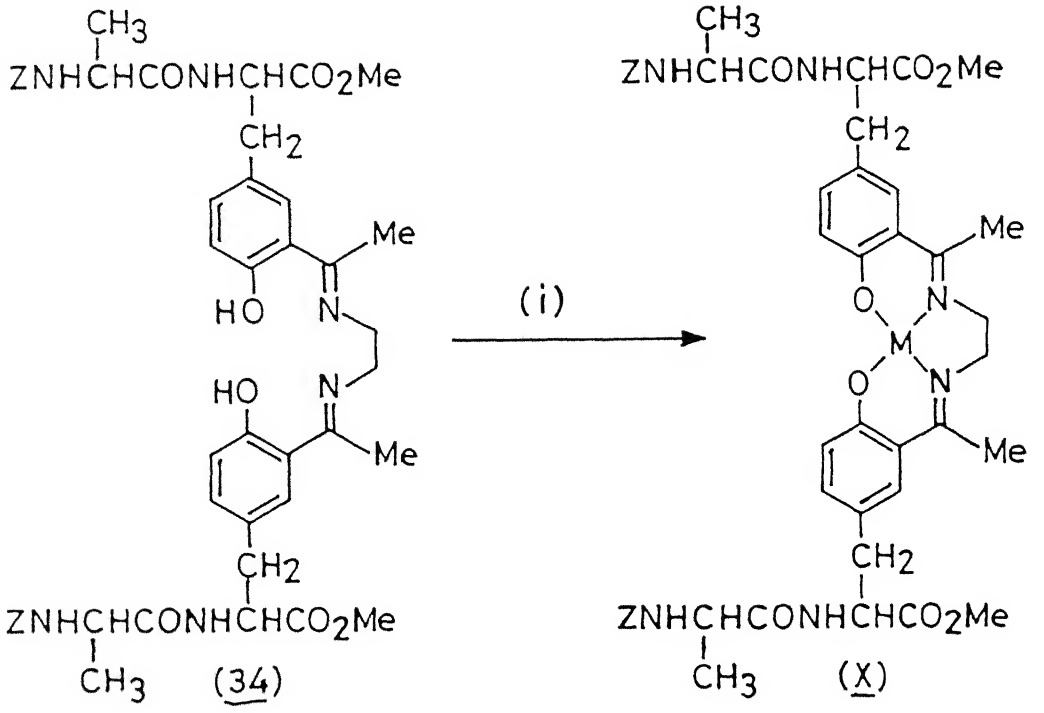
yield	:	78%
mp	:	115-119°C
ir(KBr)ν_{max} cm^{-1}	:	3321, 2927, 1718, 1662, 1614, 1587, 1530
epr($CHCl_3$, -196°C)	:	$A_{\|\|} = 205$, $g_1 = 2.198$, $g_2 = 2.045$, $g_3 = 1.992$
ms (m/z)	:	971 (MH)$^+$

(67)

yield	:	78%
mp	:	221-223°C (dec.)
ir(KBr)ν_{max} cm^{-1}	:	3313, 2925, 1741, 1693, 1654, 1616 1579, 1531

The EPR profile of (66) at LNT was very good thus enabling the estimation of the appropriate parameters (*vide supra*) which showed it to be a square planar complex. Of interest is the observation that the mass spectrum of (66) exhibited base peak corresponding to the parent ion at 971 demonstrating the stability of this compound.

CHART C.I.25



M	(X)
Cu(II)	(66)
Ni(II)	(67)

(i) $M(OAc)_2$ –MeOH , 1h

Similarly, the dipeptide composite namely, ZSerTyr(3-Ac)OMe- ethylenediamine composite (36), readily afforded Cu(II) (68), Ni(II) (69) and Co(II) (70) complexes (CHART C.I.26).

---

(68)

yield	:	82%
mp	:	168-169°C
ir(KBr)ν_{max} cm^{-1}	:	3326, 2928, 2850, 1742, 1626, 1575
epr($CHCl_3$, -196°C)	:	$A_{\|\|} = 200$, $g_1 = 2.198$, $g_2 = 2.066$, $g_3 = 1.986$

(69)

yield	:	71%
mp	:	139°C
ir(KBr)ν_{max} cm^{-1}	:	3313, 2926, 1734, 1654, 1615, 1531
ms (m/z)	:	998 $(MH)^+$

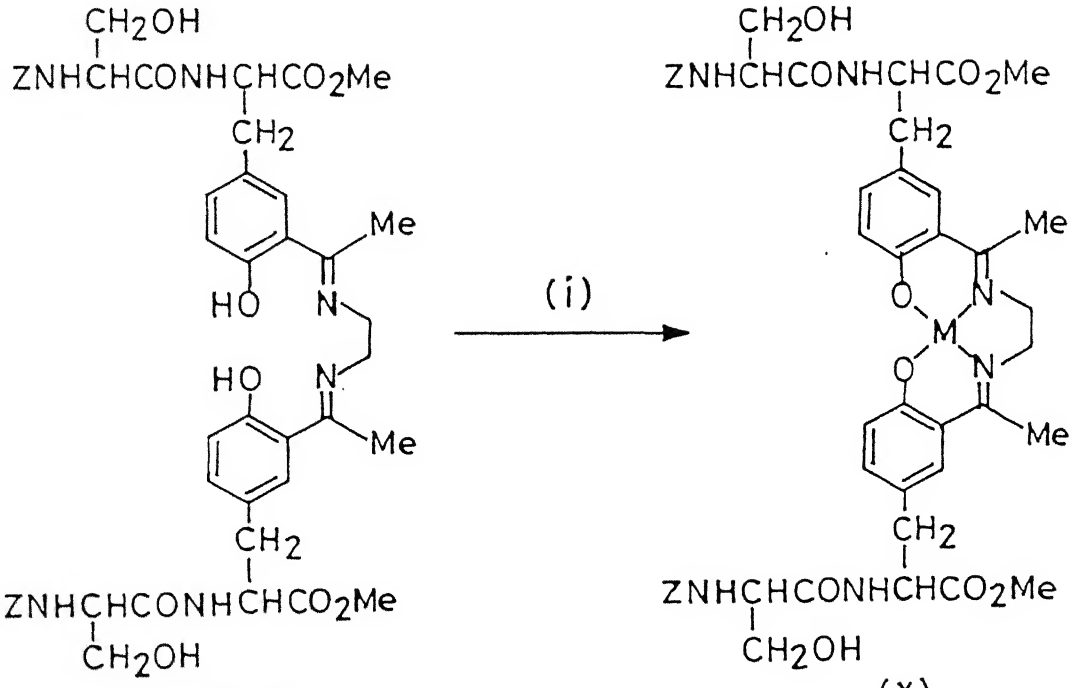
(70)

yield	:	76%
mp	:	126-129°C
ir(KBr)ν_{max} cm^{-1}	:	3312, 2928, 1748, 1690, 1648, 1540
uv-vis (DMSO)λ_{max} nm	:	260, 326, 403, 556

Compound (68) as in the previous cases afforded an excellent EPR profile (*vide supra*). Compound (69) exhibited in the FAB mass spectrum a strong peak at 998 corresponding to the parent ion.

ZAlaTyr(3-Ac)SerOMe - ethylenediamine composite (35), where the template is in a truly peptide environment, readily afforded the Cu(II) template (71) (CHART C.I.27).

CHART C.I.26

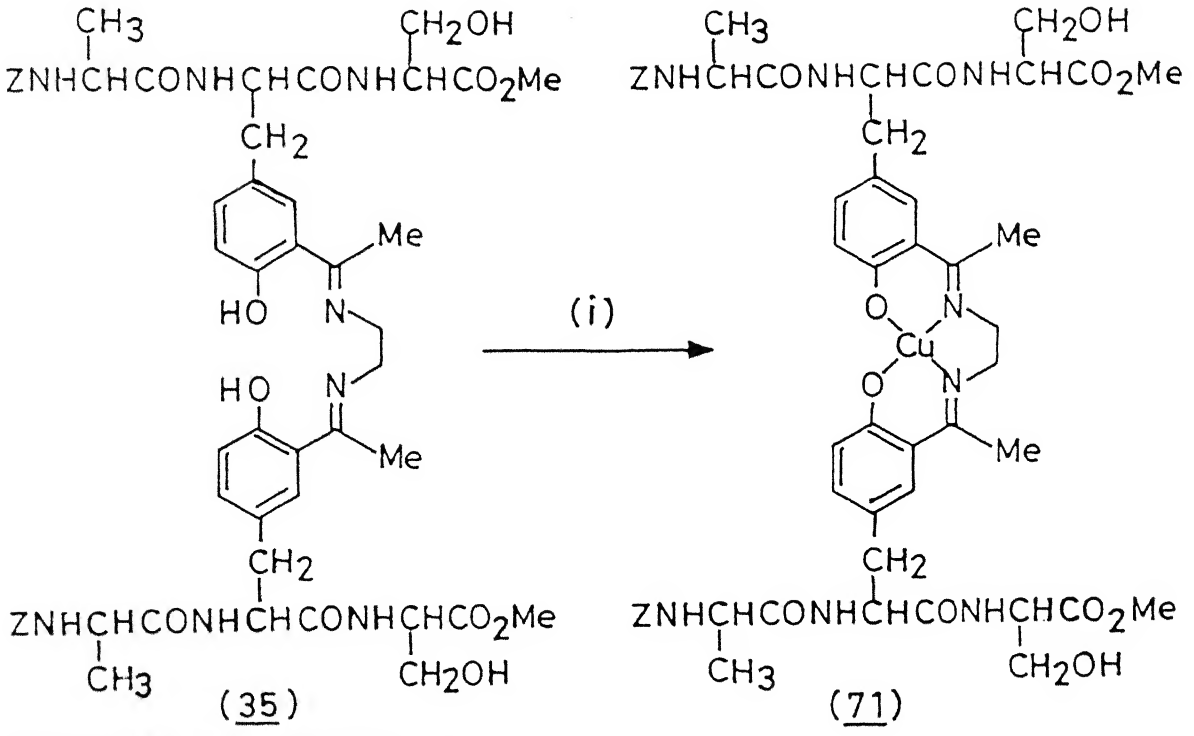


(36) (X)

M	(X)
Cu(II)	(68)
Ni(II)	(69)
Co(II)	(70)

(i) $M(OAc)_2$–MeOH, 1h

CHART C.I.27



(35) (71)

(i) $Cu(OAc)_2$ – MeOH, 1h

(71)

yield	:	87%
mp	:	219-220°C
ir(KBr)ν_{max} cm^{-1}	:	3303, 2926, 1744, 1653, 1588, 1532
epr($CHCl_3$, -196°C)	:	$A_{\|\|} = 200$, $g_1 = 2.205$, $g_2 = 2.043$, $g_3 = 1.991$
ms (m/z)	:	1167 $(MH+Na)^+$, 1145 $(MH)^+$
uv-vis (DMSO)λ_{max} nm	:	262, 368, 559

Compound (71) showed an excellent EPR profile at LNT (*vide supra*) and in FAB mass spectrum exhibited peak at 1145 corresponding to the parent ion (MH) as well as peak at 1167 corresponding to Na ion uptake.

In the early stages of the work the optimum condition for template formation with AEH and its subsequent metal uptake were worked out on the logical model salicylaldehyde (72). Thus a solution of (72) underwent smooth condensation with AEH in MeOH at room temperature for 1 h to afford the template (73), which on treatment with copper acetate and nickel acetate in MeOH for 1 h afforded the expected templates (74) and (75) in excellent yields (CHART C.I.28).

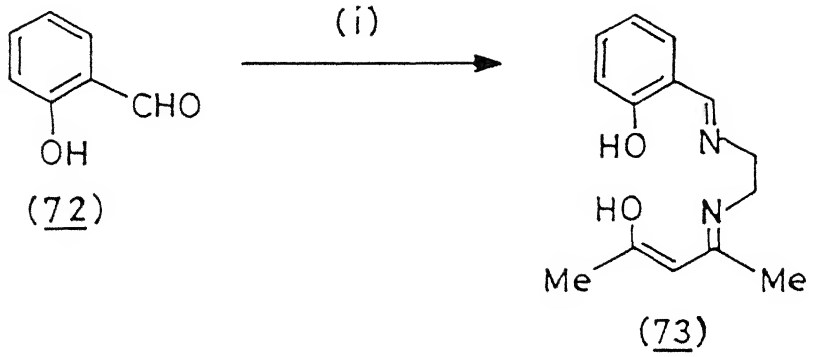
(73)

yield	:	79%
mp	:	67°C
ir(KBr)ν_{max} cm^{-1}	:	3394, 2992, 2945, 1740, 1631, 1607, 1569
nmr($CDCl_3$) δ	:	1.97 (d, 6H, CH_3 x 2), 3.75 (m, 4H, $-CH_2CH_2-$), 4.98 (s, 1H, enolic CH), 6.78-7.50 (m, 4H, aromatic), 8.4 (s, 1H, -N=CH), 10.97 (s, 1H, enolic OH), 12.98 (s, 1H, phenolic OH)
ms (m/z)	:	247 $(MH)^+$

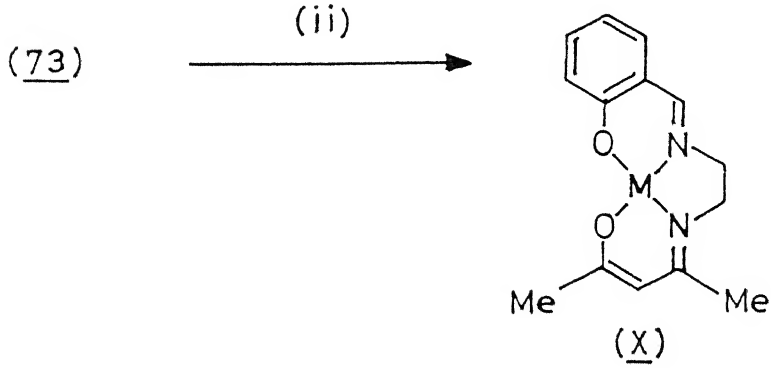
(74)

yield	:	86%
mp	:	178°C

CHART C.I.28



(72) (73)



(X)

M	(X)
Cu(II)	(74)
Ni(II)	(75)

(i) AEH (11) – MeOH, 2h

(ii) $M(OAc)_2$ – MeOH, 1h

ir(KBr)ν_{max} cm^{-1}	:	3420, 2919, 1631, 1599, 1532, 1511
epr(MeOH, rt)	:	$A_{iso} = 90$, $g_{iso} = 2.1107$
(MeOH,-196°C)	:	$A_{\|\|} = 195$, $g_1 = 2.217$, $g_2 = 2.044$, $g_3 = 1.998$

(75)

yield	:	88%
mp	:	217°C
ir(KBr)ν_{max} cm^{-1}	:	3420, 2924, 1623, 1599, 1536, 1512

A comparison of the NMR spectra of (73) with that derived from BzTyr(3-Ac)OMe - AEH adduct (12) showed remarkable similarities which was expected. A surprising feature however is the appearance of the phenolic proton at 15.37 ppm in the case of (12) compared to 12.98 with (73). Compound (73) afforded an exceptionally clean FAB mass spectrum, where the base peak at 247 corresponding to $(MH)^+$ was by for the most preponderant. Interestingly, a minor peak could also be seen at 269 corresponding to uptake of Na ion.

The objective of the present endeavours, namely, to craft peptide segments with Cu(II) uptake potential has been successfully demonstrated.

It would now be logical to compare the profile of copper templates constructed in the present work with that of the naturally occurring copper enzymes in terms of ligand environment on the basis of $A_{\|}$, $g_{\|}$ and $g_{\perp}$ parameters. This has been done in TABLE C.I.1.

As TABLE C.I.1 would show, the corresponding properties between the synthetic and naturally occurring ones are quite remarkable.

The work described so far has shown that a metal uptake environment could be

TABLE C.I.1.

Compounds*/ Proteins	$A_{\parallel}$ (Gauss)	$g_{\parallel}$	$g_{\perp}$	ref.
(37)	95	2.112	2.013	This work
(40)	100	2.115	2.005	This work
(42)	100	2.114	2.013	This work
(44)	90	2.111	2.016	This work
(47)	100	2.112	2.011	This work
(50)	95	2.111	2.013	This work
(53)	100	2.116	2.016	This work
(64)	90	2.110	2.017	This work
(65)	90	2.109	2.009	This work
Stellacyanin	35	2.287	2.051	49, 50, 51
Umecyanin	35	2.317	2.050	52
Azurin	60	2.260	2.050	53
Plastocyanin	63	2.226	2.053	54
Laccase	90	2.190	2.030	49, 50, 51

★ EPR spectra recorded at room temperature

readily accomplished in peptides wherever Tyr(3-Ac) residues are present. The task now would be the construction of peptides and proteins having Tyr(3-Ac) side chains. In small peptides (30-40 residues) this would not be a problem, since methodologies worked out here would readily enable the preparation of these compounds in gram amounts by routine synthetic procedures. Alternate, more biochemically oriented, methodologies have to be explored for the incorporation of Tyr(3-Ac) residues in large proteins. It is obvious that direct acylation of tyrosine residues in peptides would be highly impractical because of interfering residues.

In recent times ingenious approaches have been made to incorporate non coded amino acids at specific sites using the ribosomal translation machinary. The most successful in this direction to date is by the use of suppressor $tRNA^s$ crafted from the three termination codons. The task here would involve, *inter alia*, the construction of a totally synthetic tRNA which would have an anticodon arm corresponding to any one of the three termination codons. This can be illustrated in the transformation of AAG anticodon arm of yeast phenylalanine tRNA (see SCHEME C.I.2). By a combination of chemical and biochemical procedures, this anticodon was transformed to AUC which would be complementary to the codon UAG, which signifies normally the termination of translation. The suppressor tRNA - suppressor in the sense that it prevents the release of the growing peptide chain - can now be used as the vehicle to incorporate a non coded amino acid at a predetermined termination codon site. This is by no means a trivial task, in the sense that the attachment of the desired amino acid residue at the 3' end of the suppressor tRNA has to be accomplished in vitro using chemical and biochemical procedures. In spite of such great challenges several non-coded amino acids have been incorporated in a site specific manner in ribosomal directed protein synthesis. These include $\alpha\alpha'$-disubstituted amino acids, N-alkyl amino acids and lactic acid. Other more precise protocols but involving greater complexity, are also available. At the other end of the spectrum is the loading of unnatural amino acids which have similarities to a coded

one.[55] In sum, an obvious way to introduce Tyr(3-Ac) unit into specific sites in proteins would be the development of methodologies for the effective loading of this non coded amino acid into suppressor tRNA. Endeavours in this direction are being explored.

It is interesting to note that once the Tyr(3-Ac) units are in position, the condensation with agents described here, particularly, AEH should not pose any problem since none of the coded amino acids harbors a carbonyl function.

Although peripheral to the goals of the present work the demonstrated ability of these templates to pick up Co(II) and Ni(II) ions is also of considerable interest. Here the cobalt systems are more significant since this metal plays pivotal and unusual roles in many biological transformations.

The extremely ready Friedel-Crafts acylation of tyrosine made it logical to explore the introduction into the tyrosine system alternate ligands, by electrophilic substitution which could directly form metal templates, a property which as stated earlier Tyr(3-Ac) did not possess. In this context, the introduction of the nitro group appeared quite attractive since the ortho nitro phenol thus created can be expected to possess metal uptake properties.

Tyrosine was nitrated using aq.HNO_3 to afford Tyr(3-Nitro) (76) which was transformed to Tyr(3-Nitro)OMe (77) (MeOH-$SOCl_2$) and then to BocTyr(3-Nitro)OMe (78) (Boc-azide, Pyridine). However, all efforts to generate metal complexes from (78) did not succeed (CHART C.I.29).

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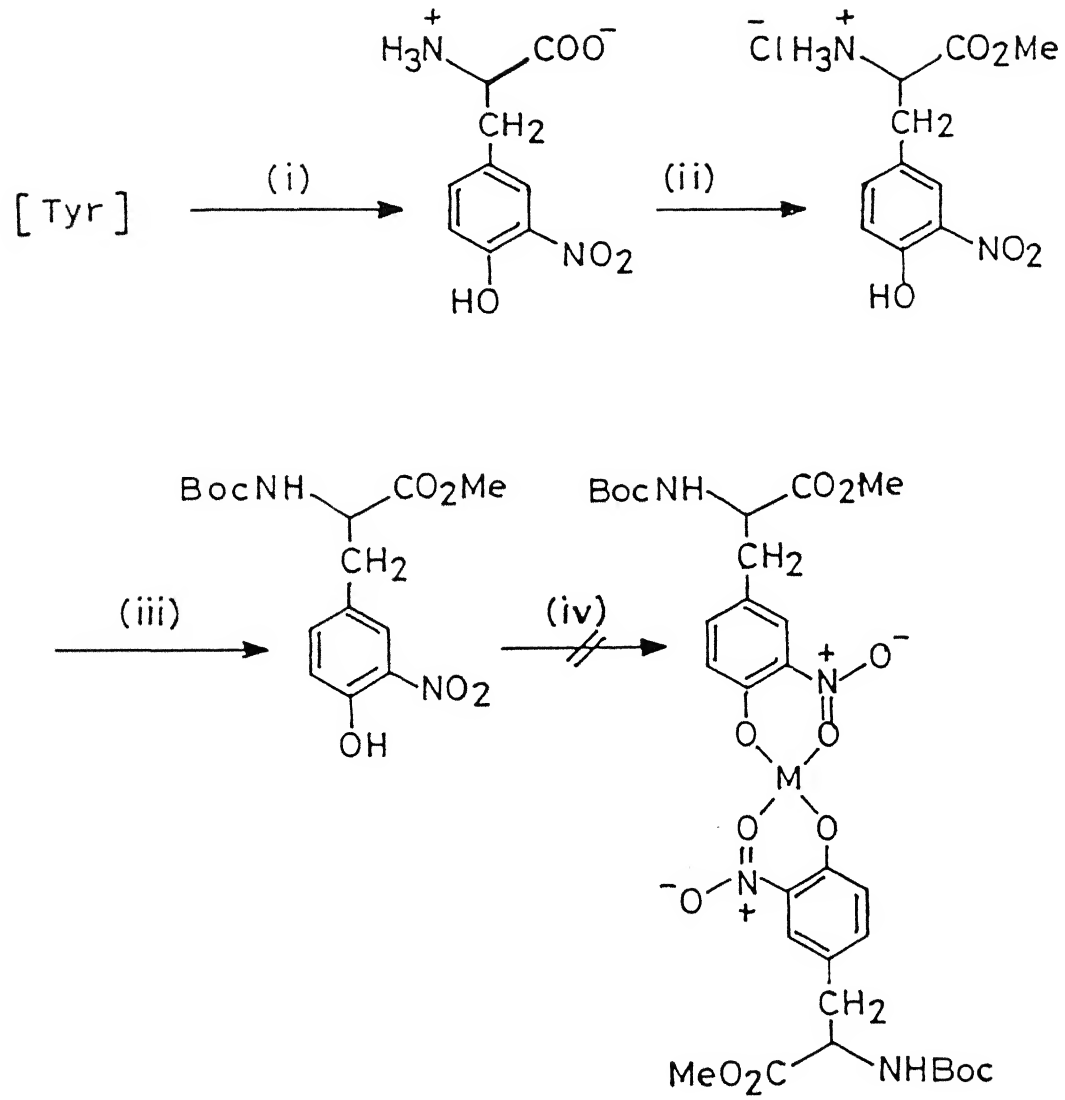
(76)

yield	:	74%
mp	:	231°C (lit[56]. mp 233-235°)
ir(KBr)ν_{max} cm^{-1}	:	3253, 2924, 1694, 1634, 1608, 1581, 1552, 1510, 1336

CHART C.I.29



(i) HNO_3-H_2O, NH_4OH

(ii) $MeOH-SOCl_2$

(iii) Boc azide–Py

M = Cu / Ni

C.II. THE TRANSFORMATION OF L-3,4-DIHYDROXYPHENYLALANINE (L-DOPA) TO CONSTRUCTS OF POSSIBLE USE IN PEPTIDE DESIGN.

The work reported in this section owes its genesis to the successful demonstration of the coded amino acid tyrosine to one having properties for metal uptake potential and at the same time could be readily incorporated into peptides. The focus of the present study is on L-3,4-dihdroxyphenylalanine (L-DOPA).

In spite of the fact that L-DOPA has found extensive therapeutic applications during the past several years, particularly with respect to the management of Parkinsonism, it has been long recognised that it is not the most ideal drug due to many reasons. The magnitude of the problem here can be recognised from the fact that this orally administered substance has to reach the brain which involves several hurdles, overcoming of which results in great loss and, in addition, produces harmful metabolites. The degradation of DOPA begins even in the gastrointestinal tract and this process continues during its passage to the liver and into the blood stream. Even in the blood stream it is continuously metabolized since the compound is unprotected either by complexation or by binding to proteins. Thus, the retardation of the metabolic degradation of DOPA and its efficient transport to the target site has been the focus of the DOPA research for several years. These have resulted in dramatic improvements, although these have not solved many of the problems related to the dissolution - adsorption - metabolism processes. Perhaps central to this issue is the paucity pertaining to the chemistry of L-DOPA. This aspect has been highlighted in SECTION-B. Consequently the major thrust of the work in this section is aimed at possible addition of novel chemical transformations involving L-DOPA, that could enhance the therapeutic potential of DOPA. Another equally important objective was the generation of constructs from L-DOPA which in a protein environment could lead to highly organised structures. The goals that were set

in the initial phase of the investigation here are illustrated in SCHEME C.II.1.

The ready transformation of Tyr to Tyr(3-Ac) made the preparation of the corresponding compound from DOPA namely 5-Acetyl-DOPA [DOPA(5-Ac)] extremely attractive. The hydroxyl groups in DOPA(5-Ac) will be chemically non equivalent because of strong hydrogen bonding of 4-hydroxyl group to the proximate acyl group. Thus it was envisaged that ionophores arms could be easily attached to 3-hydroxyl unit. The resulting compound as could be readily seen from SCHEME C.II.1 is extremely attractive since highly stabilised bi-metallic clusters could be easily generated by ethylenediamine condensation and complexation. In the event these transformations could be experimentally realised and modified N,C-protection could be incorporated, analogous compounds wherein the crucial α-amino acid grouping is present in a bi-metallic complexed environment could be secured. Leastwise, the metabolic fates of such compounds during passage starting from the gastrointestinal tract would be a very worthwhile study.

Experimental endeavours in this direction had as the primary focus the introduction of the crucial 5-acetyl substituent. In the event, as shown in CHART C.II.1, this could not be realised in spite of many attempts. Thus, the reaction of DOPA with $AcCl/AlCl_3$ - which was so effective in the case of Tyr - failed. Although disappointing this was not totally unanticipated since it is known that unlike phenol, catechol is extremely resistant to electrophilic substitution. In the present case the recalcitrant behaviour of DOPA towards Friedel-Crafts acylation can be explained as shown in CHART C.II.1, via formation of highly electrophilic aluminium complexes, promoted by the vicinal hydroxy groups present.

As shown in CHART C.II.2, nitration of DOPA under usual conditions failed.

Thus it was clear that optimum chemistry had to be developed to secure the synthetic objective and for this purpose N,C-protected DOPA was considered as the logical starting

SCHEME C.II.1

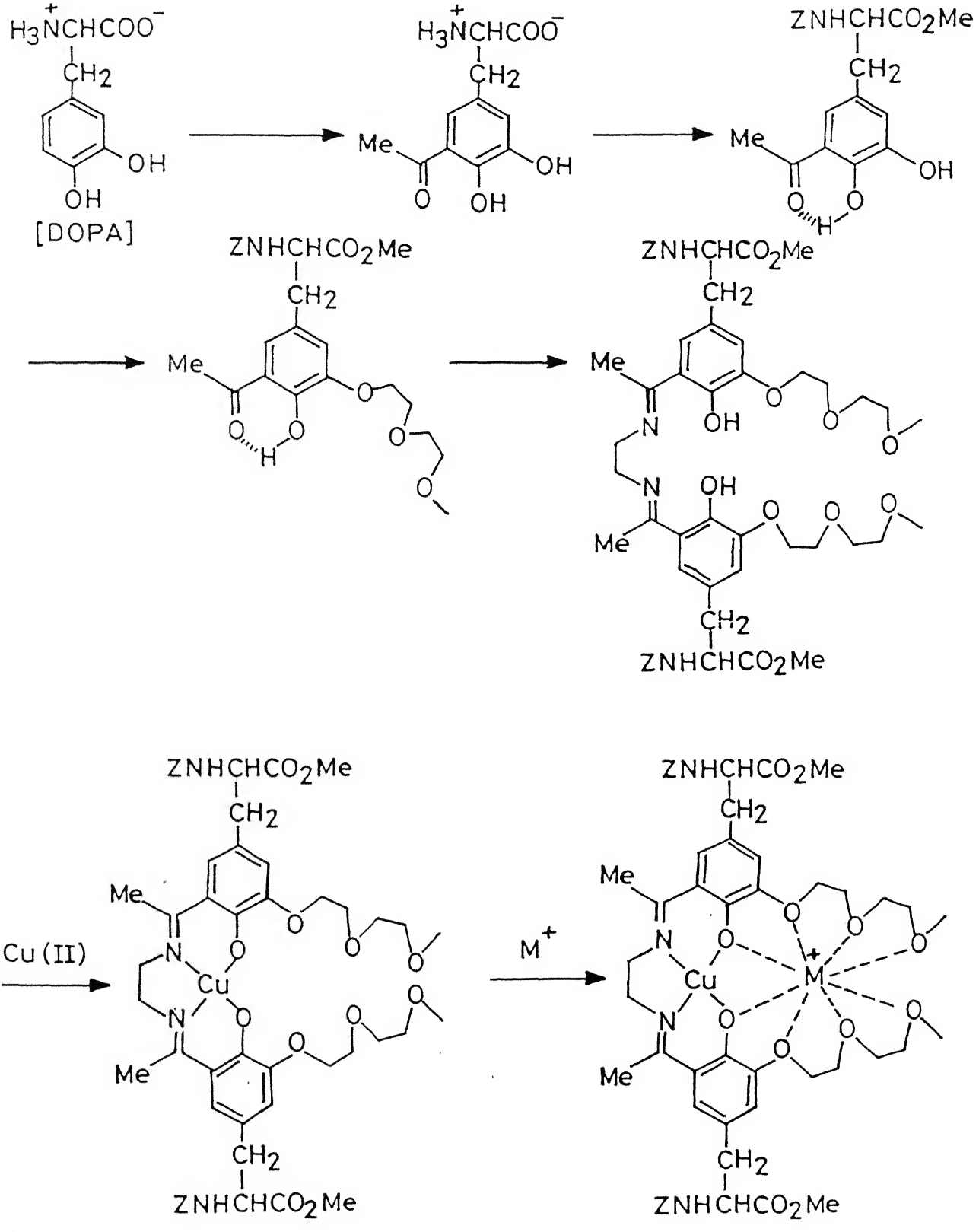
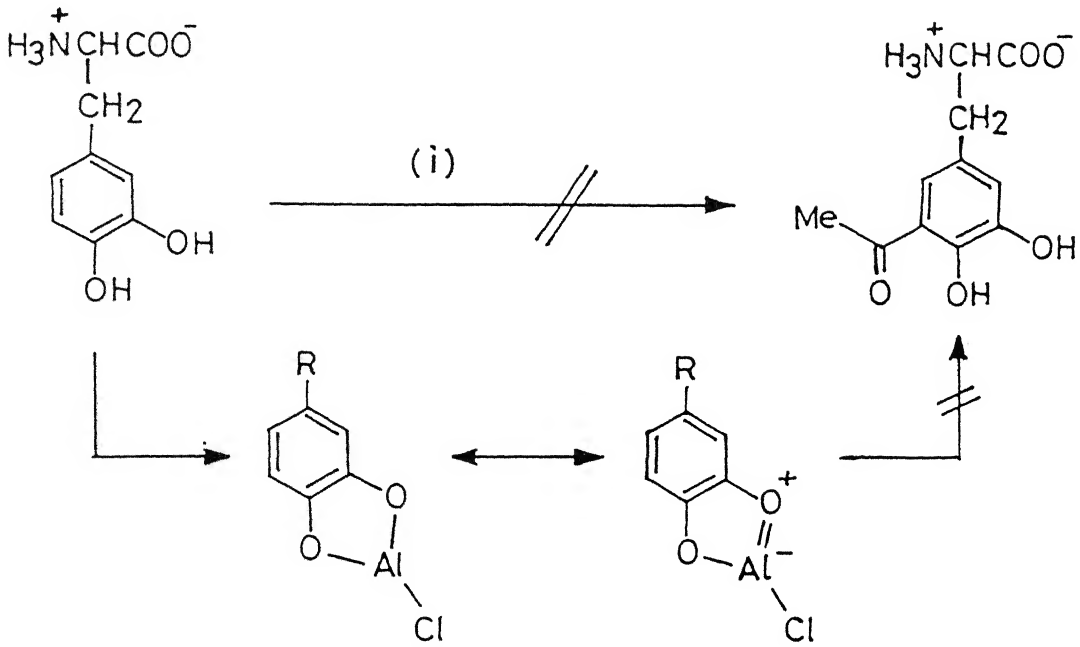
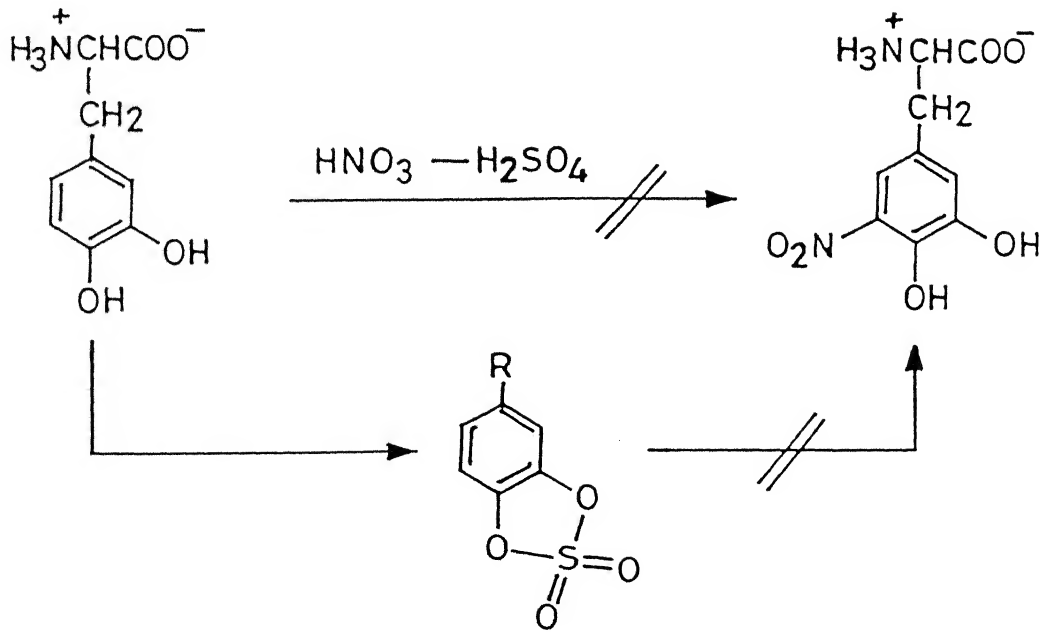


CHART C.II.1



(i) $AlCl_3$, AcCl, $PhNO_2$, RT/100°, 15h

CHART C.II.2



material.

The reaction of L-DOPA with methanolic HCl afforded DOPA-OMe.HCl (79) which was N-formylated in presence of HCOOH, CH_3COONa and Ac_2O to afford N-formyl-DOPA-OMe (80). Alternately, N-protection was readily achieved either using Z-Cl or Bz-Cl to afford Z-DOPA-OMe (81) and Bz-DOPA-OMe (82) (CHART C.II.3).

---

(79)

yield	:	85%
mp	:	174 °C (lit.[58] mp 170-171°C)
ir (KBr) ν_{max} cm^{-1}	:	3406(br), 1742, 1611, 1528, 1446
nmr(D_2O) δ	:	3.06 (d, 2H, $C^{\beta}H_2$), 3.72 (s, 3H, $COOCH_3$), 4.25 (t, 1H, $C^{\alpha}H$), 6.69 (m, 3H, aromatic)

(80)

yield	:	95%
mp	:	123° C (lit.[8] mp 119-121°C)
ir(KBr)ν_{max} cm^{-1}	:	3526, 3348, 1728, 1632, 1533, 1443
nmr($CDCl_3$-DMSO-d_6) δ	:	3.0 (d, 2H, $C^{\beta}H_2$), 3.72 (s, 3H, $COOCH_3$), 4.81 (q, 1H, $C^{\alpha}H$), 6.38-6.88 (m, 3H, aromatic), 7.06 (d, 1H, NH), 8.16 (s, 1H, CHO)
ms (m/z)	:	240 $(MH)^+$

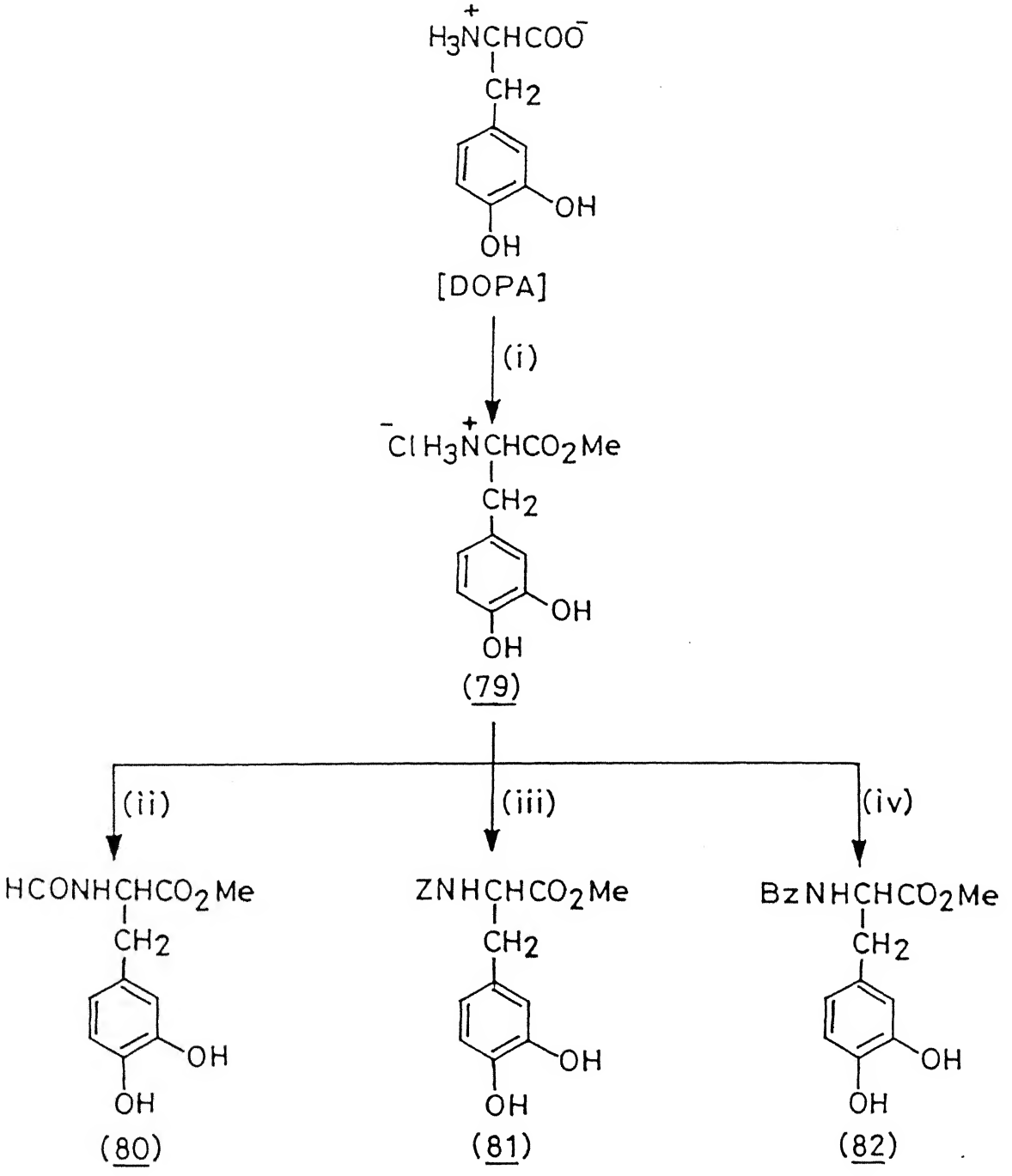
(81)

yield	:	81%
mp	:	gummy
ir (neat) ν_{max} cm^{-1}	:	3392 2956, 1714, 1610, 1520, 1445
nmr($CDCl_3$) δ	:	3.06 (d, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.66 (q, 1H, $C^{\alpha}H$), 5.09 (s, 2H, Z CH_2), 5.44 (d, 1H, NH), 6.47-6.88 (m, 3H, dopa aromatic), 7.41 (s, 5H, Z aromatic)

(82)

yield	:	81%
mp	:	72°C

CHART C.II.3



(i) MeOH-HCl (ii) HCOOH, HCOONa, Ac_2O

(iii) Z-Cl, Na_2CO_3, H_2O-Et_2O

(iv) Bz-Cl, Na_2CO_3, H_2O-Et_2O

ir (KBr) ν_{max} cm^{-1}	:	3340(br), 1720, 1628, 1586, 1562, 1505, 1472
nmr($CDCl_3$) δ	:	3.03 (d, 2H, $C^{\beta}H_2$), 3.66 (s, 3H, $COOCH_3$), 4.94 (q, 1H, $C^{\alpha}H$), 6.34-6.91 (m, 4H, NH + dopa aromatic), 7.22-7.78 (s, 5H, Bz aromatic)

CHART C.II.4 outlines endeavours to prepare the desired DOPA(5-Ac) side chain by a photo Fries rearrangement from the appropriate O-acetyl precursor. Reaction of DOPA-OMe.HCl (79) with Ac_2O-pyridine readily afforded N-protected O,O'-diacetyl compound (83). The transformation of (83) to N-acetyl-3- or 4- O-mono-acetyl-DOPA-OMe by chemical as well as enzymatic procedures did not succeed. Under most conditions as exemplified with $NaHCO_3$ in aqueous MeOH, both acetyl groups were cleaved to afford N-AcDOPA-OMe (84) (CHART C.II.4).

(83)

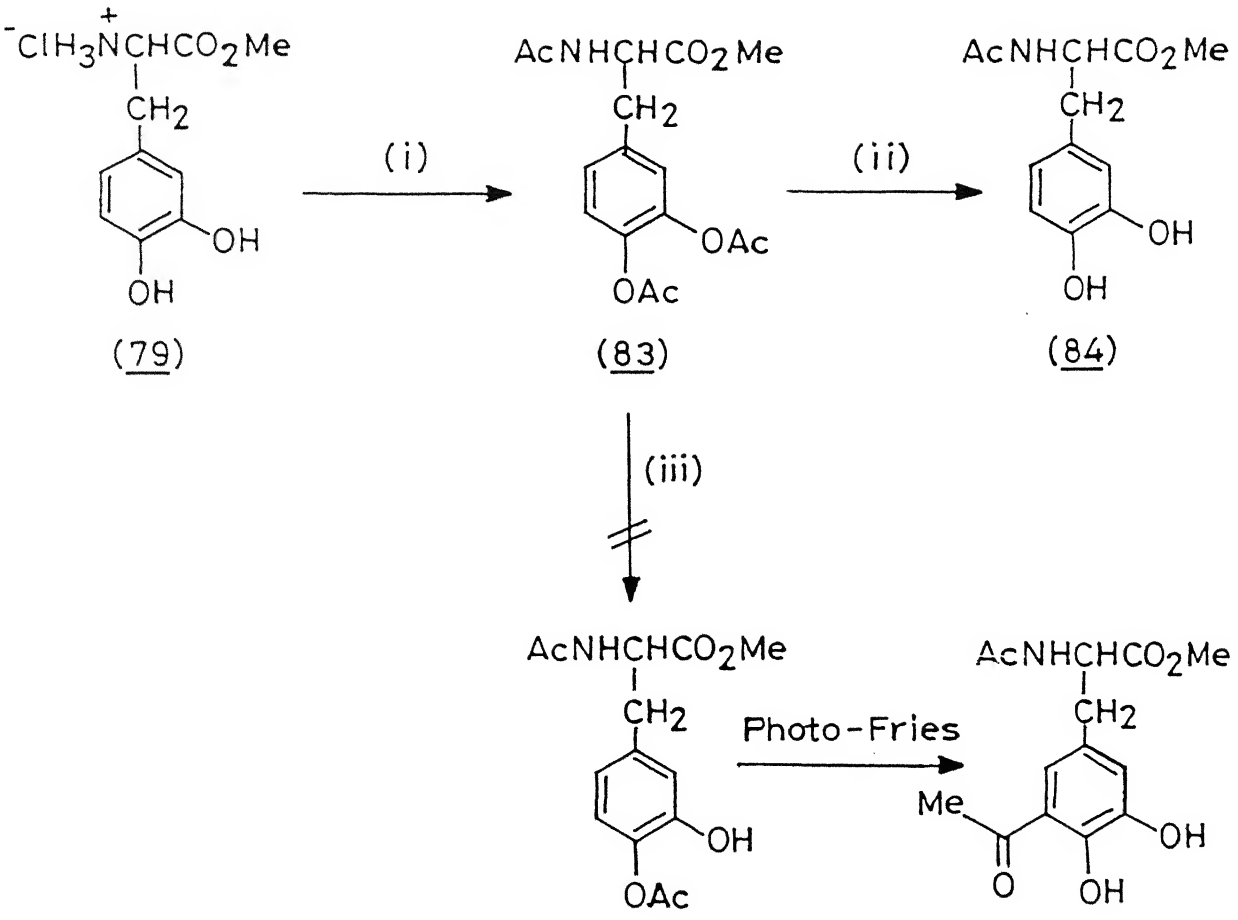
yield	:	82%
mp	:	116°C
ir (KBr) ν_{max} cm^{-1}	:	3322, 2956, 1735, 1642, 1530, 1504
nmr($CDCl_3$) δ	:	2.0 (s, 3H, N-$COCH_3$), 2.28 (s, 6H, $OCOCH_3$ × 2), 3.16 (d, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.91 (q, 1H, $C^{\alpha}H$), 6.19 (d, 1H, NH), 6.91-7.22 (m, 3H, NH, aromatic).

(84)

yield	:	75%
mp	:	gummy
ir(neat)ν_{max} cm^{-1}	:	3320(br), 1730, 1713, 1695, 1666, 1650, 1537
nmr($CDCl_3$) δ	:	1.9 (s, 3H, $NCOCH_3$), 2.92 (d, 2H, $C^{\beta}H_2$), 3.65 (s, 3H, $COOCH_3$), 4.7 (q, 1H, $C^{\alpha}H$), 5.92 (d, 1H, NH), 6.28-6.85 (m, 3H, aromatic)

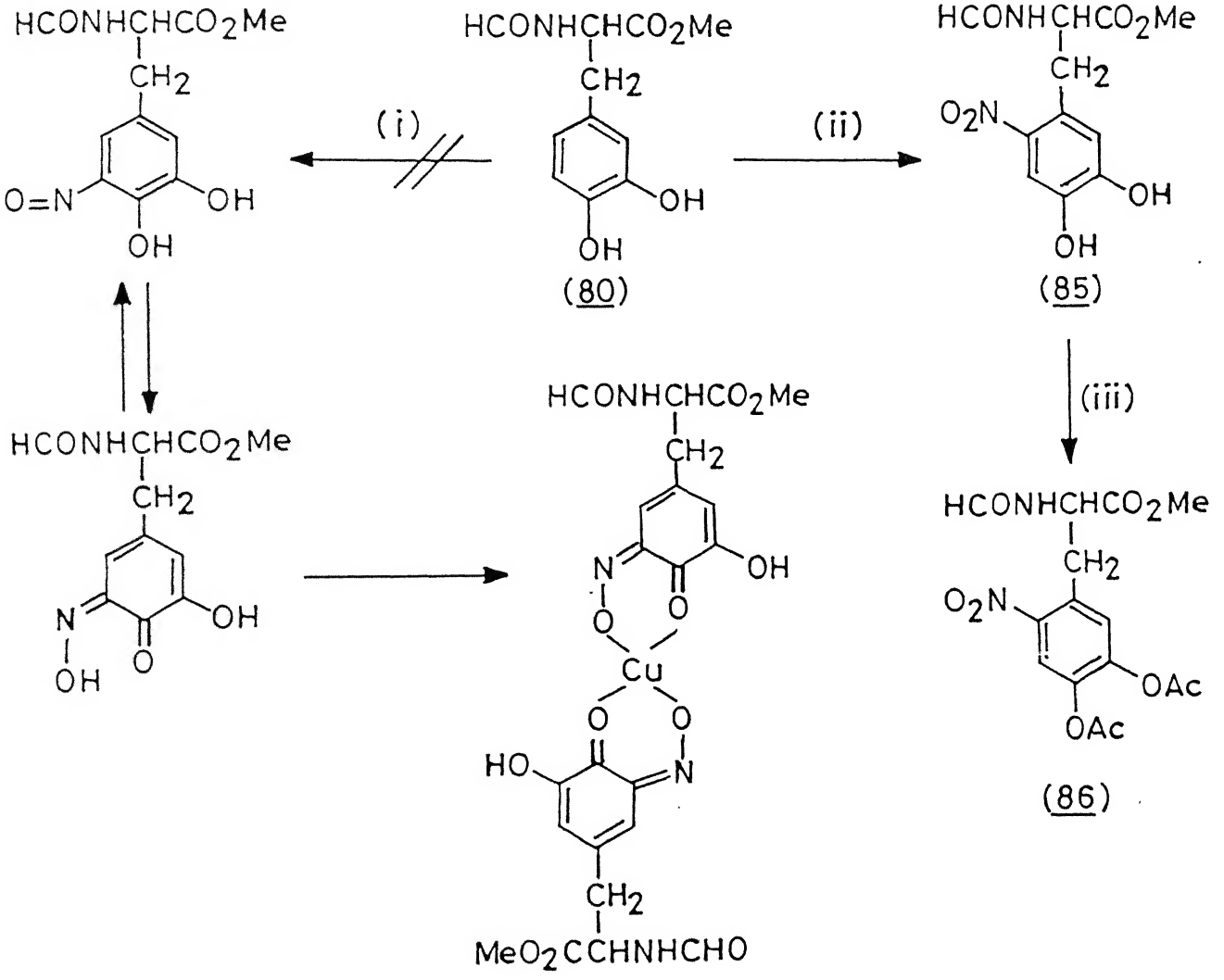
In view of the serious problems encountered in direct nitration of DOPA, alternate method, involving nitrosation and oxidation was attempted. Interestingly, as shown in CHART C.II.5 the desired nitroso compound can exhibit tautomerism with an α-oximino ketone structure which would enable formation of metal complexes. In the event however nitrosation under conditions used for phenol[59] with cupric nitrate, H_2O_2

CHART C.II.4



(i) Ac_2O – Py (ii) $NaHCO_3$, MeOH / H_2O

CHART C.II.5



(i) $Cu(NO_3)_2$, $NH_2OH{\cdot}HCl$, H_2O_2 , HCl

(ii) $NaNO_2$, dil H_2SO_4

(iii) Ac_2O , Py

and HCl led to intractable, highly colored, products. However treatment of (80) with $NaNO_2$ and H_2SO_4 afforded a crystalline mono nitro compound. Unfortunately the NMR spectrum clearly showed that it arose from the only undesirable option namely substitution at the 6-position, since the other two possibilities namely substitution at 2 or 5 could have led to compounds that could be further elaborated to target molecules. It should be noted that substitution at 2 location would have resulted in AB profile in the aromatic region of the NMR where as substitution at 5 location would have resulted in two peaks that would show the characteristic meta coupling. On the other hand substitution at 6 location would lead to two singlets which was what observed. Thus the nitro compound was assigned (85) which was further characterised by acetylation to (86) (CHART C.II.5).

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(85)

yield	:	83%
mp	:	168-169°C
ir (KBr) ν_{max} cm^{-1}	:	3403, 3322, 1720, 1651, 1597, 1537, 1334, 1308
nmr($CDCl_3$-DMSO-d_6) δ	:	2.94 -3.50 (m, 2H, $C^{\beta}H_2$), 3.72 (s, 3H, $COOCH_3$), 4.88 (m, 1H, $C^{\alpha}H$), 6.81 (s, 1H, aromatic), 7.28-7.75 (m, 2H, NH + aromatic), 8.13 (s, 1H, CHO), 7.28-7.75 (m, 2H, NH + aromatic), 8.13 (s, 1H, CHO)
ms (m/z)	:	285 $(MH)^+$)

(86)

yield	:	82%
mp	:	gummy
ir (neat) ν_{max} cm^{-1}	:	3368, 1734, 1654, 1597, 1534, 1331
nmr($CDCl_3$) δ	:	2.35 (s, 6H, $OCOCH_3 \times 2$), 3.54 (m, 2H, $C^{\beta}H_2$), 3.78 (s, 3H, $COOCH_3$), 5.0 (q, 1H, $C^{\alpha}H$), 6.63 (d, 1H, NH), 7.25 (s,1H, aromatic), 7.89 (s, 1H, aromatic), 8.26 (s, 1H, CHO)

In an alternate approach it was considered feasible to craft a metal uptake center using one of the hydroxyl groups of DOPA as shown in CHART C.II.6. As shown here, mono-O-alkylation with bromo dimethyl malonate would lead to systems that can

be further elaborated to constructs having potential for primary and secondary metal ion uptake. Thus the alkylation of N-formyl-DOPA-OMe (80) with dimethyl bromo malonate was carried out in NaH and DMF. The expectation here was that the dianion precursor would undergo alkylation at the relatively less acidic 4-positions. In the event however the mono alkylated compound obtained in 14% yield turned out to be 3-O-alkylated product (87). The structural assignment is based on the NMR spectrum which showed significant down field shift of the 6-H compared to the parent one. This can be expected if the O-alkylation had taken place at the 3-position (CHART C.II.6).

---

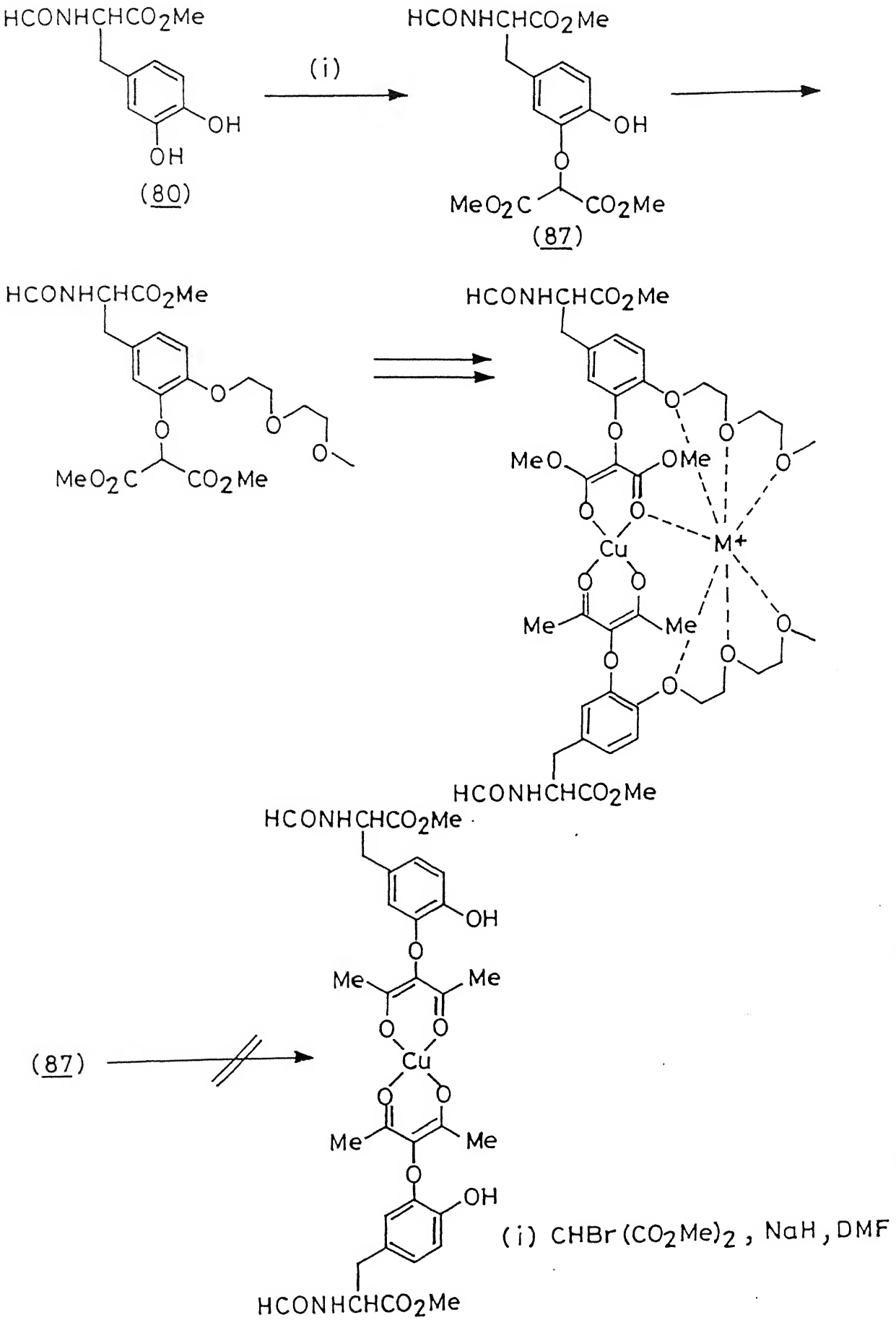
(87)

yield	:	24%
mp	:	gummy
ir (neat) ν_{max} cm^{-1}	:	3418(br), 2923, 2852, 1733, 1684, 1652, 1558, 1506
nmr($CDCl_3$) δ	:	3.05 (d, 2H, $C^{\beta}H_2$), 3.74 (s, 3H, dopa ester), 3.85 (s, 6H, malonic $COOCH_3$ × 2), 4.32 (m, 1H, malonic CH), 4.89 (q, 1H, $C^{\alpha}H$), 6.1 (d, 1H, NH), 6.58-6.9 (m,3H, aromatic), 7.1 (s, 1H, phenolic OH), 8.09 (s, 1H, CHO)
ms (m/z)	:	369 $(M)^+$

Attempts to form metal complexes with (87) did not succeed. Therefore, further work along the lines shown in CHART C.II.6 was not pursued. Nevertheless compound (87), although obtained in poor yields would be an interesting substrate pertaining to the preparation of diverse derivatives of DOPA. One of which would be the attachment of uracil moiety by treatment with urea.

The difficulties encountered in the introduction of the ring substituents in DOPA led to the notion that the use of the existing vicinal dihydroxy groups to prepare constructs having metal uptake profile appeared more practical. At the outset it was considered expedient to generate ionophores from DOPA by simple substitution with the MEM (methoxy ethoxy methyl) group. Since this unit can be easily introduced and the resulting systems are highly stable to a variety of reagents. In the event, treatment of

CHART C.II.6


HCONHCHCO2Me
OH
OH
(80)
(i)
HCONHCHCO2Me
OH
O
MeO2C
CO2Me
(87)
HCONHCHCO2Me
O
O
O
MeO2C
CO2Me
HCONHCHCO2Me
MeO
OMe
Cu
M+
Me
Me
HCONHCHCO2Me
(87)
HCONHCHCO2Me
OH
Me
Me
Cu
Me
Me
OH
HCONHCHCO2Me
(i) CHBr(CO2Me)2 , NaH , DMF

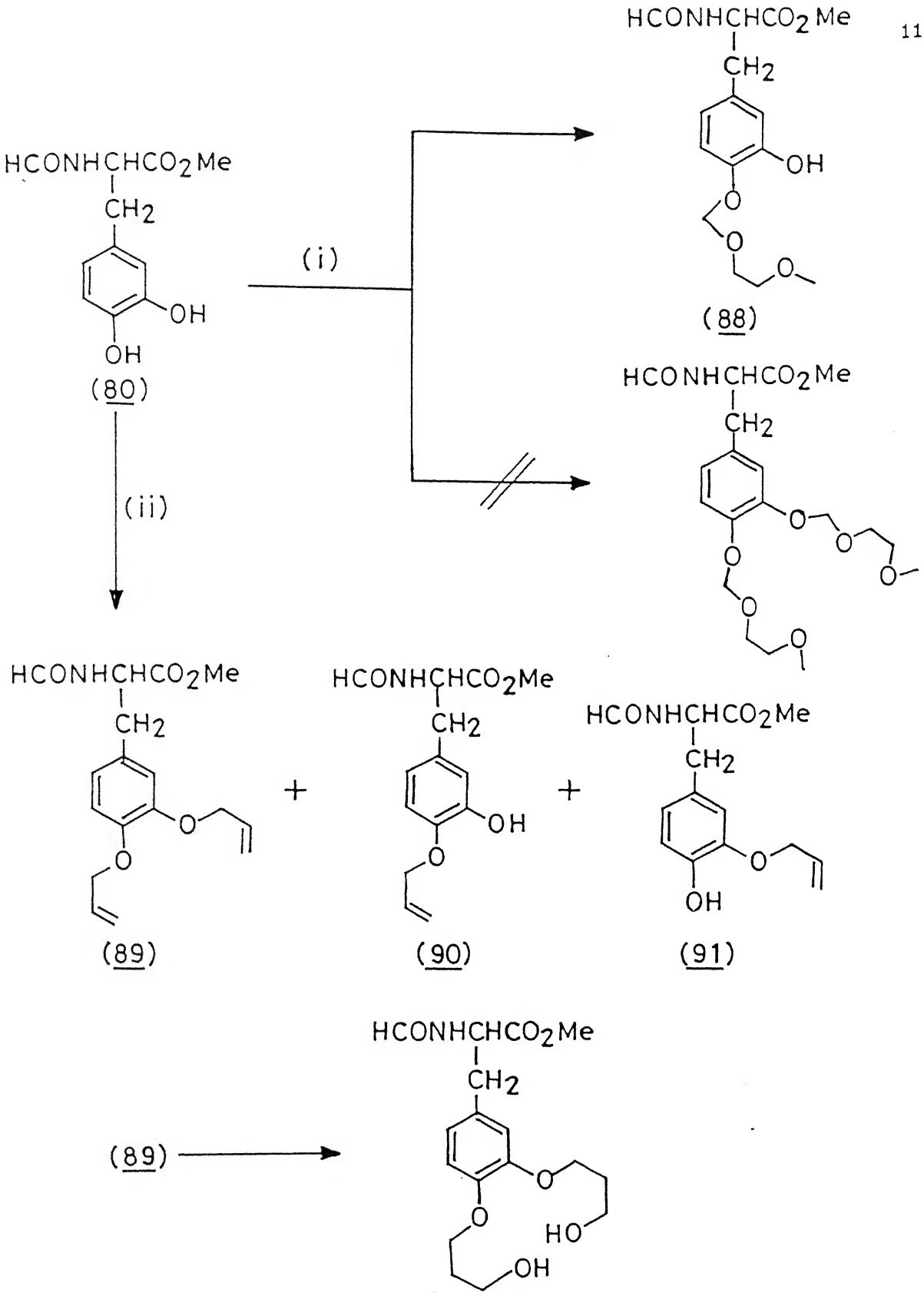
N-formyl-DOPA-OMe (80) with excess MEM chloride in aqueous Na_2CO_3 afforded only the monoprotected derivative which on the basis of its NMR, particularly on the basis of the resemblance of the aromatic protons to that of the parent compound (80), the 4-alkylated structure (88) has been assigned. The structural assignment has been supported by the FAB mass spectrum which exhibited peak at 328 corresponding to the parent ion $(MH)^+$. The absence of the desired bis-alkylation was rather surprising and could arise because of the strong complexation of the sodium salt of the 3-hydroxyl group, greatly assisted by stabilisation from mono alkylated semi crown system. An alternate strategy to overcome this problem involved possible bis alkylation of DOPA, since, as shown in CHART C.II.7, such a system could be transformed to alcohols by hydroboration reactions. The resulting diols could themselves exhibit metal uptake properties and could be further elaborated to crown ethers by alkylation.

In the event reaction of N-formyl-DOPA-OMe (80) with excess allyl bromide in K_2CO_3-DMF afforded 3 pure products namely, the desired N-formyl-DOPA(bis-allyl)-OMe (89) (8.5% yield), N-formyl-DOPA(4-O-allyl)-OMe (90) (30% yield), and N-formyl-DOPA(3-O-allyl)-OMe (91) (20% yield). The structural assignment to (89), (90) and (91) are based on NMR and mass spectral data (CHART C.II.7).

---

(88)

yield	:	48%
mp	:	gummy
ir (neat) ν_{max} cm^{-1}	:	3340(br), 2950, 1731, 1652, 1594, 1510, 1439
nmr($CDCl_3$) δ	:	3.03 (d, 2H, $C^{\beta}H_2$), 3.44 (s, 3H, OCH_3), 3.53-3.81 (m, 7H, $-CH_2-CH_2-$ + $COOCH_3$), 4.69-4.97 (m, 3H, OCH_2O + $C^{\alpha}H$), 6.5 (d, 1H, aromatic), 6.7 (s, 1H, aromatic), 6.81 (d, 1H, aromatic), 7.03 (d, 1H, NH), 8.19 (s, 1H, CHO).
ms (m/z)	:	328 $(MH)^+$



(i) MEM Chloride, aq. Na_2CO_3

(ii) Allyl bromide, K_2CO_3, DMF

(89)

yield	:	8.5%
mp	:	gummy
ir (neat) ν_{max} cm^{-1}	:	3314(br), 2924, 2856, 1744, 1683, 1513, 1425
nmr($CDCl_3$) δ	:	2.95 (d, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.43 (d, 4H, OCH_2 × 2), 4.79 (q, 1H, $C^{\alpha}H$), 5.0-5.5 (m, 4H, $=CH_2$ × 2), 5.66-6.23 (m, 3H, NH + =CH- × 2), 6.36-6.80 (m, 3H, aromatic), 7.96 (s, 1H, CHO)
ms (m/z)	:	319 $(M)^+$

(90)

yield	:	30%
mp	:	gummy
ir (neat) ν_{max} cm^{-1}	:	3340(br), 2925, 1742, 1672, 1599, 1514, 1437
nmr($CDCl_3$) δ	:	3.09 (d, 2H, $C^{\beta}H_2$), 3.78 (s, 3H, $COOCH_3$), 4.59 (d, 2H, OCH_2), 4.97 (q, 1H, $C^{\alpha}H$), 5.19-5.63 (m, 2H, $=CH_2$), 5.81-6.44 (m, 3H, NH + =CH- + ar C-6 H), 6.53-6.97 (m, 2H, ar C-2 H + ar C-5 H), 8.22 (s, 1H, CHO)
ms (m/z)	:	280 $(MH)^+$

(91)

yield	:	20%
mp	:	gummy
ir (neat) ν_{max} cm^{-1}	:	3342(br), 3028, 2928, 2953, 1742, 1672, 1595, 1513
nmr($CDCl_3$)δ	:	3.06 (d, 2H, $C^{\beta}H_2$), 3.69 (s, 3H, $COOCH_3$), 4.56 (d, 2H, OCH_2), 4.94 (q, 1H, $C^{\alpha}H$), 5.19-5.59 (m, 2H, $=CH_2$), 5.84-6.31 (m, 2H, NH + =CH-), 6.41-7.0 (m, 3H, aromatic), 8.25 (s, 1H, CHO)
ms (m/z)	:	280 $(MH)^+$

A noteworthy feature of FAB mass spectrum of (89) was the presence of, in addition to the base peak at 319 corresponding to parent ion $(M)^+$, a peak at 639 $(M_2H)^+$ corresponding to the dimeric structure. It can readily be seen from CHART C.II.7, that compound (89) could produce ordered structures by stacking interactions promoted by the side chain and the presence of dimeric peak in the FAB mass spectrum tend to support this notion.

The work reported in the present section demonstrates, above all, that chemical transformations involving DOPA, as attested by the literature information (SECTION.B) is difficult. In spite of this, the work reported here has led to the synthesis of several lead molecules notable amongst which are compounds (87) and (89).

C.III. SYNTHETIC ROUTES TO SULPHUR BRIDGED BI-METALLIC CLUSTERS FROM 3-ACETYL-TYROSINE - CYS TINE COMPOSITES AND CARBONYL LINKED CYSTINYL CYSTINE

The work presented in SECTION.C.I has amply illustrated the potential of 3-acetyl-tyrosine in the formation of metal templates of biological relevance. It was logical, therefore, to explore possibilities for using this system to craft templates that can accommodate more than one metal center. As a starting point designs were envisaged that could incorporate two metal ions, thus forming the nucleus for the construction of bi-metallic clusters. The impetus to proceed in this direction came from successful endeavours from this laboratory[60] to craft templates which can accommodate a tetrahedrally co-ordinated Zn(II) system.

In the very recent past, tetrahedrally co-ordinated Zn(II), crafted into the manifold of protein architecture has emerged as a versatile, flexible and aesthetically pleasing motif, which, by virtu of its ligand disposition, is adept in not only steering the protein ensemble to perform its orchestrated role pertaining to interaction with information, but also in striking the first note related to DNA recognition. The ubiquitous and maverick profile of templates crafted around Zn(II) is increasingly becoming obvious. To date three classes of Zn templates have been recognised as agents that play a pivotal role in DNA sequence recognition. These are the Zn finger motif, wherein a Zn(II) ion placed in a $(thiolate)_2(imidazole)_2$ environment successfully pilot peptide recognition system to proper sites in DNA, a prerequisite for initiation of transcription. A second important category pertains to Zn(II) templates placed in $(thiolate)_4$ environment. These play a critical role in the manifestation of hormone action leading to protein synthesis directed by the information system. Thus glucocorticoids, steroids and retinoids turn on the gene present in the cell nucleus by complexation with receptor proteins located in the cell surface, movement of the composite to nucleus, recognition of the specific

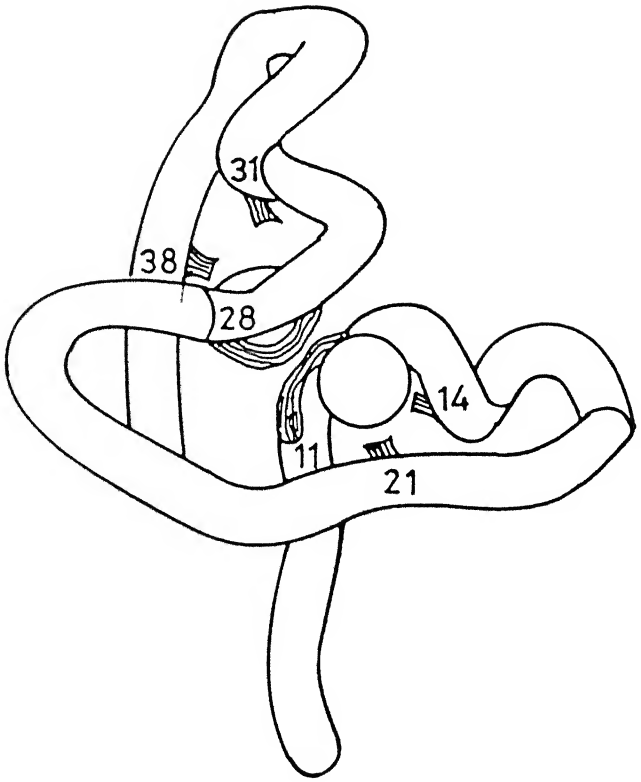
sequence and promote transcription. Crystallographic study has shown that the recognition helix in glucocorticoid receptor protein is preceded and followed by Zn template motifs, each arising from co-ordination with four invariable cysteines. Perhaps the most complex of such motifs recognised to date is the one associated with the production of enzymes that can metabolise galactose and mellibiose to biologically compatible high energy compounds. The 881 amino acid units protein - GAL4 - activates transcription of genes using several autonomous regions responsible for specific function which are essential for the production of appropriate messenger RNA necessary for translation. The initiation of transcription requires the recognition of 17 base pairs by binding of the protein as a dimer. The DNA recognition is achieved by the first 64 residues. Independent dimerisation is brought about by the 65-94 segment.

Recent crystallographic studies of a specific DNA complex of the 65 residues recognition fragment have shown that it consists of a compact metal binding domain (8-40), an extended linker (41-49) and an α-helical dimerisation element (50-64). The structure of the metal binding DNA recognition module of the GAL4 protein is presented in SCHEME C.III.1a and the details of the metal co-ordination in SCHEME C.III.1b. Thus, an approximately 30 residues protein is able to generate a bi-metallic Zn cluster involving 6 cysteines wherein the thiol groupings at positions 11 and 28 are shared by the Zn ions. The remaining tetrahedral co-ordination sites being provided by the thiol groupings at 14 and 21 locations with respect to one Zn and 31 and 38 with respect to other.

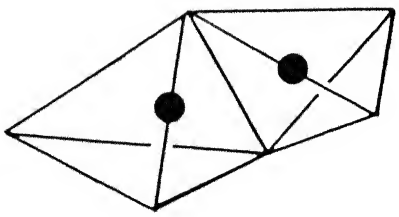
Interestingly, the co-ordination profile presented in SCHEME C.III.1a, which directly recognises a DNA triplet sequence has a profile similar to metallothioneins,[61] whose general picture is presented in SCHEME C.III.1c.

The crafting of peptide segments having metal uptake potential, which, in principle, could replace large autonomous regions found in enzymes to achieve this objective, from

SCHEME C.III.1



(a)



(b)

$$\left[Zn_2 (SR)_6 \right]^{2-}$$

(c)

3-acetyl-tyrosine, made it attractive to explore the use of this construct to craft Zn clusters which have a profile similar to that presented in SCHEME C.III.1a, with respect to the positioning of two bridge sulphur atoms co-ordinated to the two metal sites. That, such an objective could be readily achieved is illustrated in SCHEME C.III.2. Thus, neutral Zn clusters of the profile $[Zn_2(SR)_2(OR)_2(NX_3)_2]$ could be prepared via Schiff base formation of 3-acetyl tyrosine with β-amino thiols followed by metal complexation.

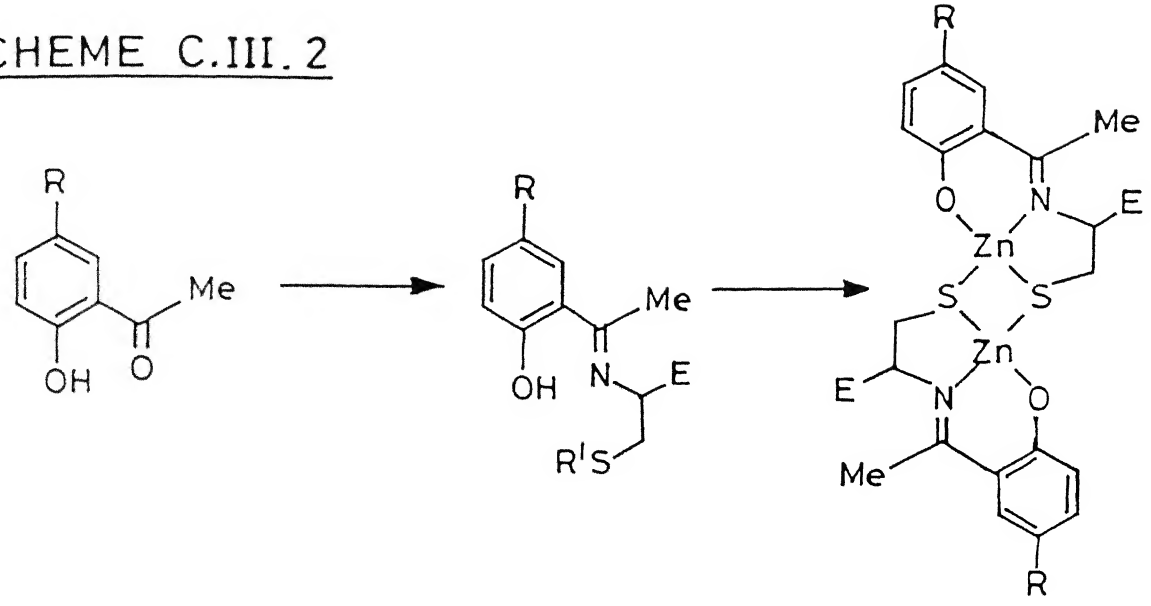
The readily available cystine-di-OMe could be easily adapted to SCHEME C.III.2, as shown in CHART C.III.1. In the event all efforts to prepare the Schiff base from ZTyr(3-Ac)OMe (4) and cystine-diOMe failed. In another more direct approach S-acetamido-methyl-Cys-OMe was reacted with (4). As could be seen from CHART C.III.2 the anticipated Schiff base on sulphur deprotection and complexation could lead to the desired Zn clusters. In the event this reaction also did not succeed.

As described in SECTION.C.I, a range of compounds having a primary amino functionality readily undergoes Schiff base formation with (4) even at room temperature. The only reasonable explanation for the failure of the formation of Schiff base either with cystine-diOMe or with S-acetamidomethyl-Cys-OMe is that, these having the amino group in a secondary carbon, makes the transition state for Schiff base formation sterically crowded.

It was considered that the 3-formyl analog of (4) would offer a better chance for Schiff base formation with either cystine-diOMe or S-acetamidomethyl-Cys-OMe. Unfortunately desired compound is not reported and likely pathways[14] that could lead to the compound appeared to be experimentally cumbersome.

The replacement of the nitrogen and oxygen ligands in the cluster profile envisaged in SCHEME C.III.2 with sulphur can be expected to afford bi-metallic Zn clusters of the type $[Zn_2(SR)_6]^{2-}]$ as shown in SCHEME C.III.3. Indeed the profile here would be much

SCHEME C.III.2



SCHEME C.III.3

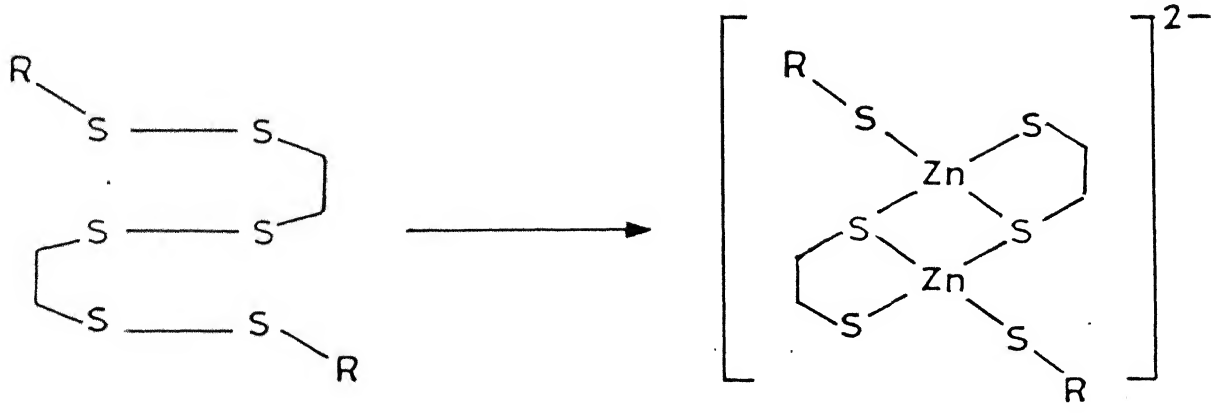


CHART C.III.1

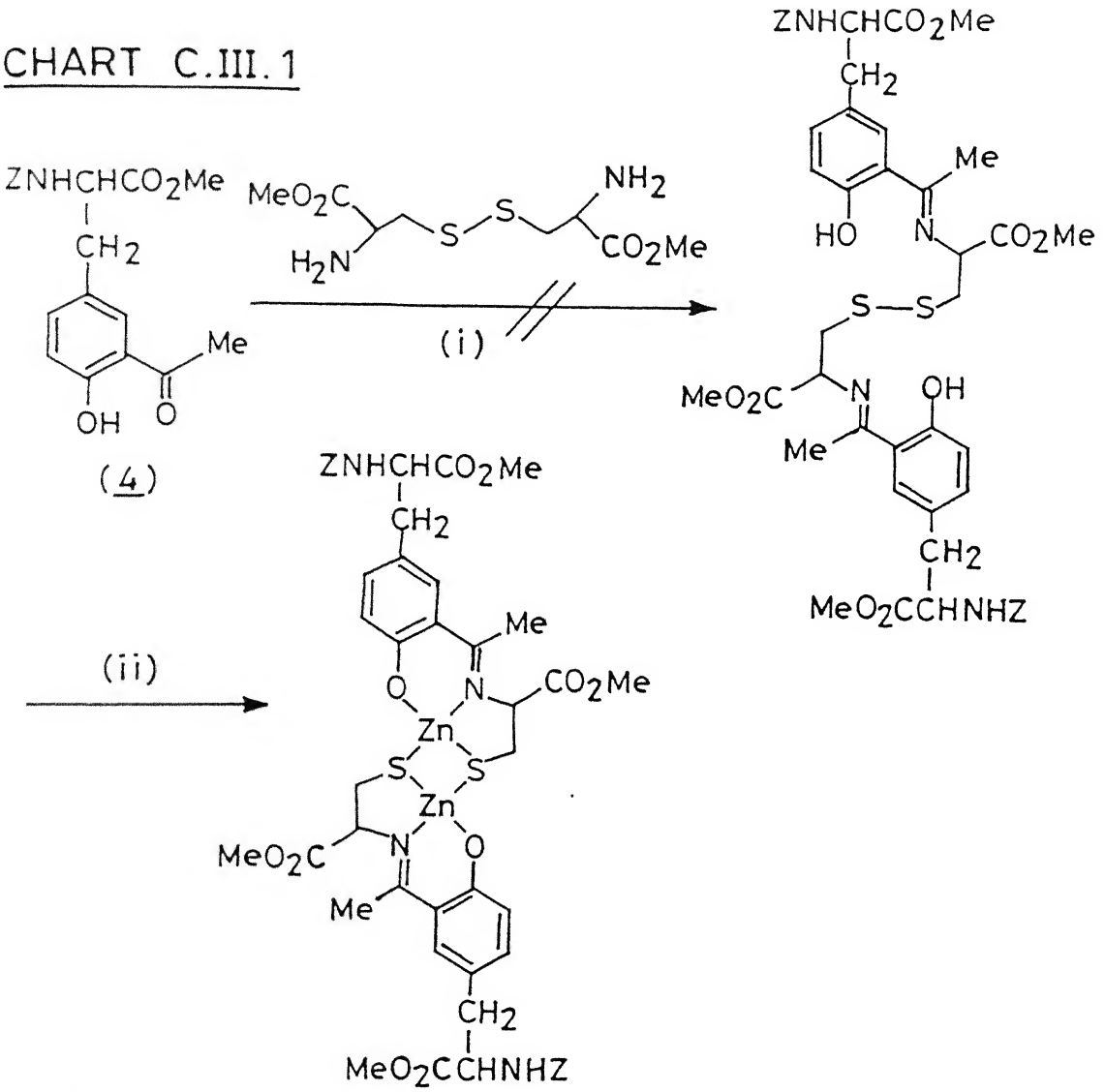
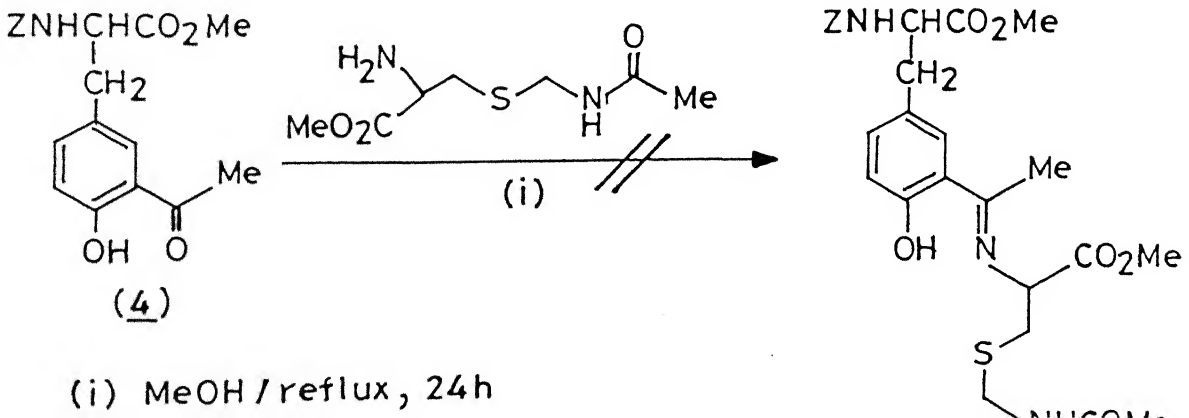


CHART C.III.2



closer to the design in the GAL4 protein in SCHEME C.III.1. As shown in SCHEME C.III.4, the required array of the disulphide pairs can be constructed by condensing, in tandem, three cystines. This strategy has led to the synthesis of novel compounds (96) and (97) (*vide supra*).

Mono Z-protection of cystine was achieved by treatment with limited Z-Cl in presence of aqueous NaOH, and followed by adjustment of the pH to 3.2 when the desired mono Z-protected cystine (92) precipitates out.[62] The mono protection was clearly demonstrated by NMR and FAB mass spectra.

---

(92)

yield	:	46%
mp	:	196-200°C
ir (KBr) ν_{max} cm^{-1}	:	3360(br), 3060, 1704, 1678, 1523, 1448, 1399
ms (m/z)	:	375 $(MH)^+$

Bis-N-protection of cystine was readily achieved using either Z-Cl and NaOH or Boc-carbonate and NaOH to afford, respectively, compounds (93) and (94) (CHART C.III.3).

---

(93)

yield	:	72%
mp	:	118-120°C (lit.[63] mp 114°C)
ir (neat)ν_{max} cm^{-1}	:	3333, 3033, 1694, 1586, 1530, 1455

(94)

yield	:	66%
mp	:	137-139°C (lit.[64] mp 143-145°C)
ir(neat)ν_{max} cm^{-1}	:	3368, 2988, 1743, 1718, 1680, 1509, 1410
nmr($CDCl_3$)δ	:	1.47 (ss, 18H, Boc CH_3 × 2), 3.25 (m, 4H, $C^{\beta}H_2$ × 2), 4.47 (q, 2H,$C^{\alpha}H$ × 2), 5.78 (d, 2H, NH × 2), 6.28 (s, 2H, COOH × 2)

SCHEME C.III.4

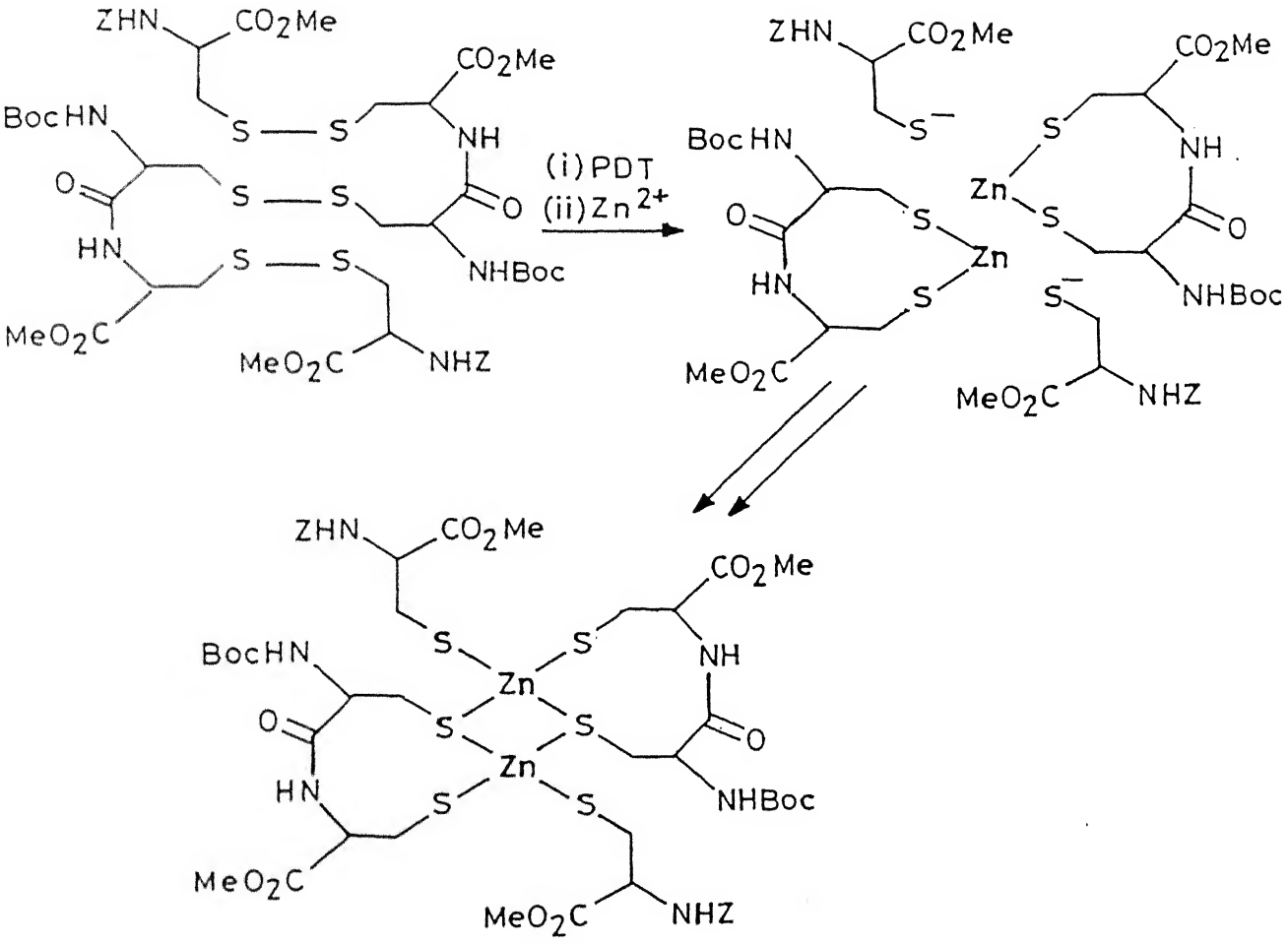
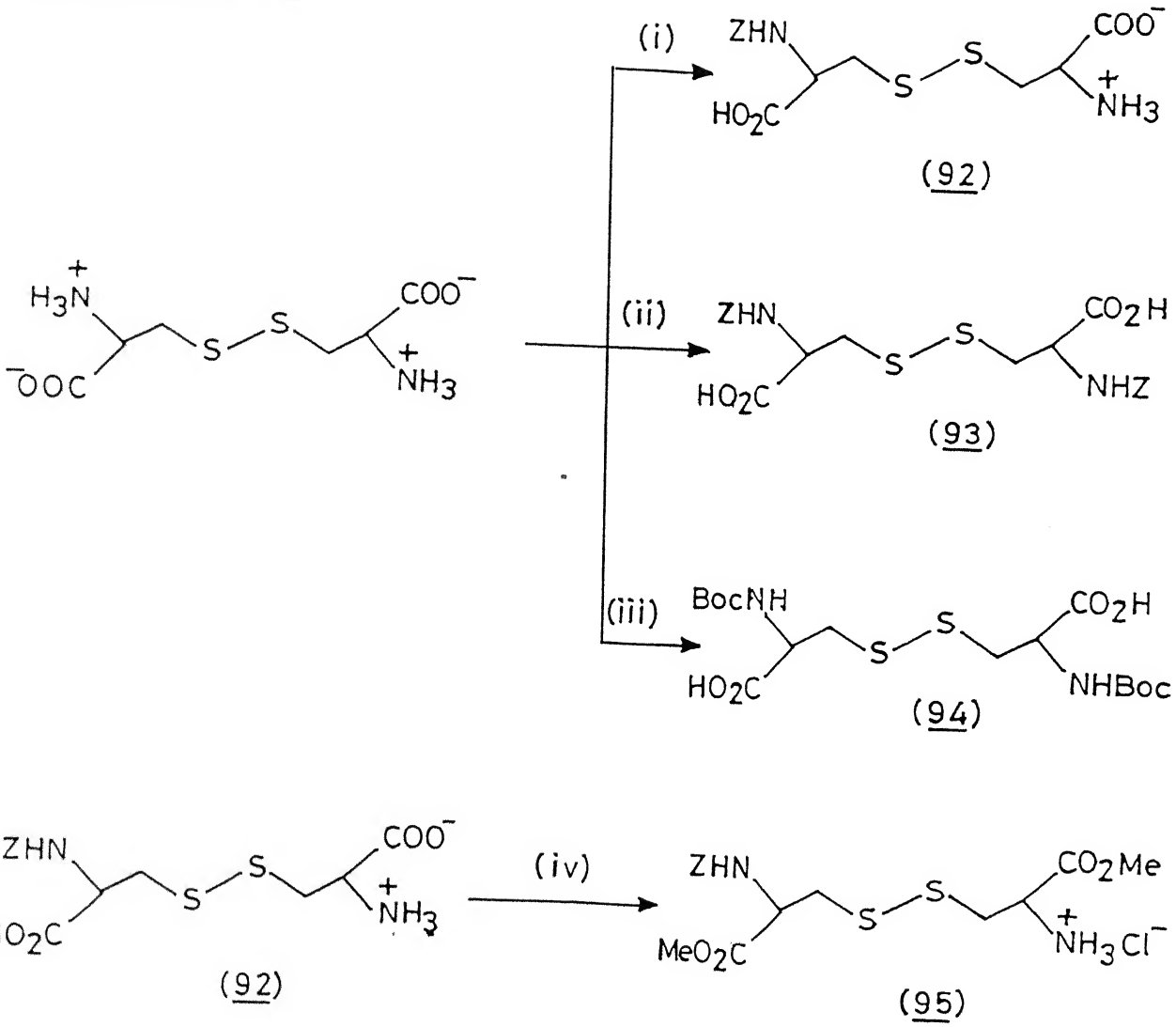


CHART C.III.3



(i) Z-Cl, NaOH, pH 3.2
(ii) Z-Cl, NaOH
(iii) Boc-Carbonate, NaOH
(iv) MeOH-HCl

Treatment of (92) with methanolic HCl afforded mono Z-protected cystine-diOMeHCl (95).

---

(95)

yield	:	76%
mp	:	149-151°C (lit.[65] mp 159-160°C)
ir(neat)ν_{max} cm^{-1}	:	3375, 2946, 2840, 1732, 1684, 1514
nmr($CDCl_3$-DMSO-d_6)δ	:	3.38 (m, 7H,NH_3^+ + $C^{\beta}H_2$ × 2), 3.81 (ss, 6H, $COOCH_3$ × 2), 4.13 - 4.69 (m, 2H, $C^{\alpha}H$ × 2), 5.13 (s, 2H, Z CH_2), 7. 34 (s, 6H, NH + Z aromatic)
ms (m/z)	:	403 $(MH)^+$ -HCl

DCC-HOBt condensation of one unit of (94) with two units (95) led to smooth bis peptidation leading to N'Z-Cystinyl(OMe) - N"N"[(bis-Boc)Cystinyl]- Cystine(N'Z)-diOMe (96) (CHART C.III.4). In a similar manner peptidation of one unit of (93) with two units of (95) afforded N'Z-Cystinyl$(OMe)_2$ - N"N"[(bis-Z)Cystinyl]- Cystine(N'Z)-diOMe (97) (CHART C.III.5).

---

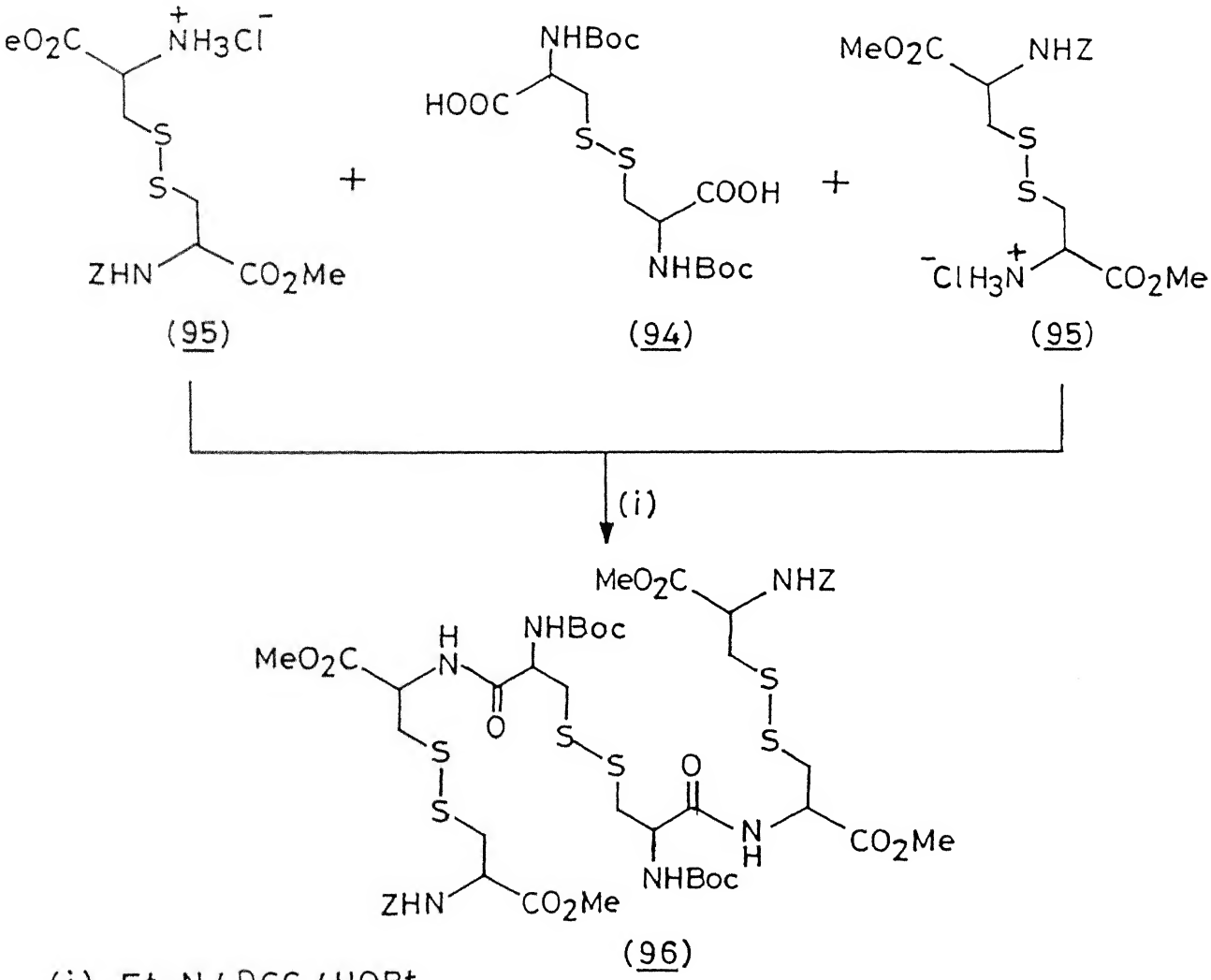
(96)

yield	:	62%
mp	:	93°C
ir(neat)ν_{max} cm^{-1}	:	3341, 2928, 1740, 1692, 1665, 1521, 1456, 1436, 1392
ms (m/z)	:	1009 $(MH)^+$ -2 Boc
uv-vis (CH_3CN)λ_{max} nm (ϵ, L mol^{-1} cm^{-1})	:	251(sh, 1527), 256(1461), 262(sh, 1223), 267(sh, 965), 325(sh, 78)

^{1}H nmr(400 MHz) studies on (96) :

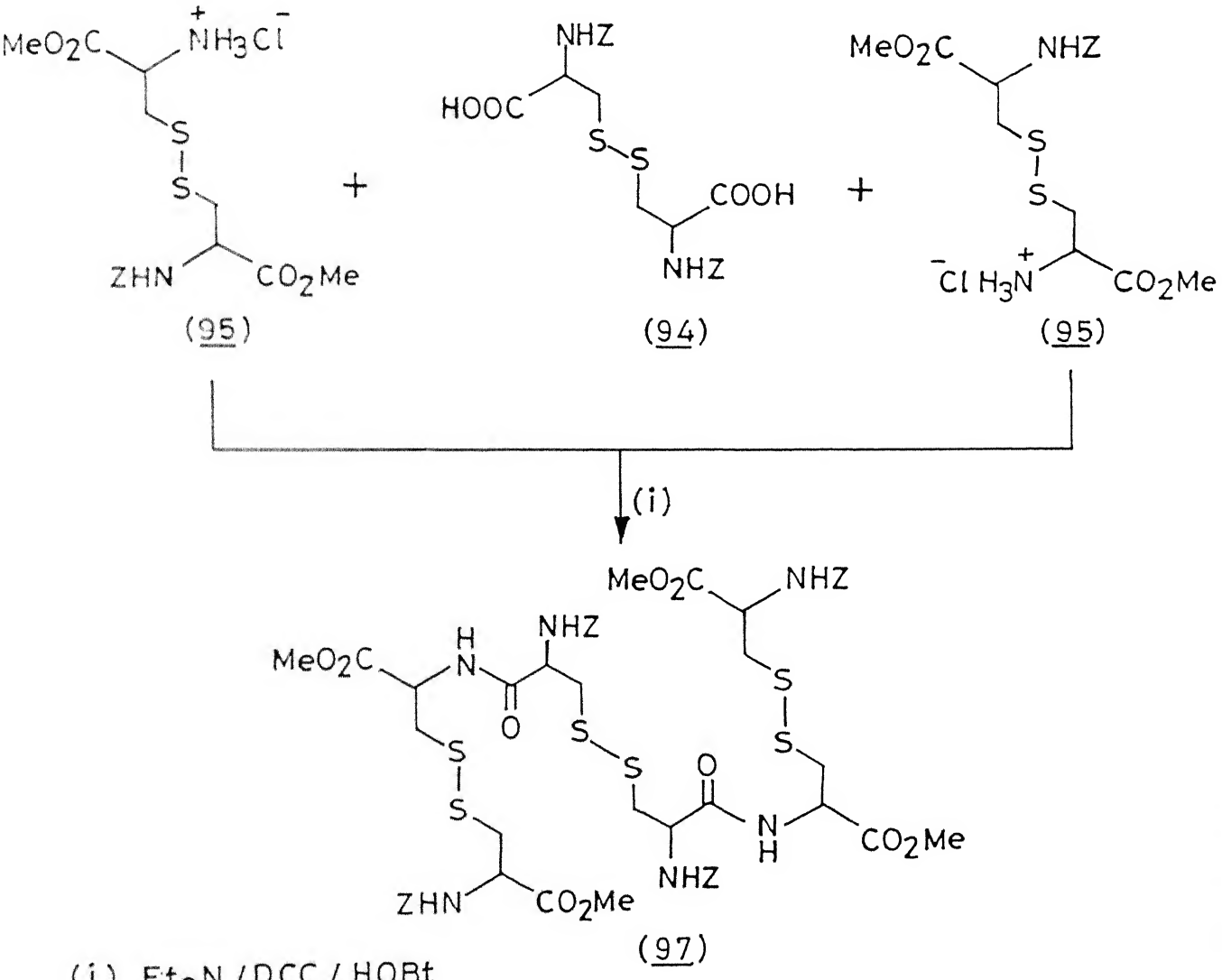
δ ($CDCl_3$), 24°C	:	1.45 (s, 18H, Boc CH_3 × 6), 3.0 (m, 4H, pep $C^{\beta}H_2$ × 2), 3.06-3.36 (m, 8H, Boc $C^{\beta}H_2$ × 2 + Z $C^{\beta}H_2$ × 2), 3.75 (m, 12H, $COOCH_3$ × 4), 4.66 (q, 2H, Z $C^{\alpha}H$ × 2), 4.79 (q, 2H, Boc $C^{\alpha}H$ × 2), 4.88 (q, 2H, pep $C^{\alpha}H$ × 2), 5.12 (ss, 4H, Z CH_2 × 2), 5.62 (d, 2H, Boc NH × 2), 5.86 (d, 2H, Z NH × 2), 7.36 (ss, 10H, aromatic), 7.85 (d, 2H, pep NH × 2)

CHART C.III.4



(i) Et_3N / DCC / HOBt

CHART C.III.5



(i) Et_3N / DCC / HOBt

δ(DMSO-d_6),24°C : 1.38 (s, 18H, Boc CH_3 × 6), 2.5 (DMSO), 2.8, 2.9 (qq, 4H, pep $C^{\beta}H_2$ × 2), 3.0 (m, 4H, Boc $C^{\beta}H_2$ × 2), 3.1 (m, 4H, Z $C^{\beta}H_2$ × 2), 3.32(H_2O), 3.65 (ss, 12H, $COOCH_3$ × 4), 4.21 (m, 2H, Boc $C^{\alpha}H$ × 2), 4.35 (m, 2H, Z $C^{\alpha}H$ × 2), 4.55 (m, 2H, pep $C^{\alpha}H$ × 2), 5.05 (s, 4H, Z CH_2 × 2), 7.05 (d, 2H, Boc NH × 2), 7.35 (s, 10H, aromatic), 7.9 (d, 2, Z NH × 2), 8.45 (d, 2H, pep NH × 2)

(97)

yield	:	52%
mp	:	127-130°C
ir(neat)ν_{max} cm^{-1}	:	3331, 2951, 1736, 1652, 1533
ms (m/z)	:	1277 $(MH)^+$
uv-vis (CH_3CN)λ_{max} nm (ϵ, L mol^{-1} cm^{-1})	:	245(sh, 2218), 251(2158), 256(2099), 262(sh, 1743), 267(sh, 1347)

^{1}H nmr(400 MHz) studies on (97) :

δ ($CDCl_3$), 24°C : 2.9 (d, 4H, pep $C^{\beta}H_2$ × 2), 3.15 (m, 8H,^{1}N $C^{\beta}H_2$ × 2 + $^{\omega}$N $C^{\beta}H_2$ × 2), 3.70 (ss, 12H, $COOCH_3$ × 4), 4.62 (q, 2H, ^{1}N $C^{\alpha}H$ × 2), 4.88 (q, 2H, pep $C^{\alpha}H$ × 2), 5.1 (ss, 8H, Z CH_2 × 4), 5.15 (dd,, 2H, $^{\omega}$N $C^{\alpha}H$ × 2), 5.8 (d, 2H, ^{1}NH × 2), 5.9 (d, 2H, $^{\omega}$NH × 2), 7.3 (m, 20H, aromatic), 8.15 (d, 2H, pep NH × 2)

δ(DMSO-d_6),24°C : 2.85 (dd, 4H, pep $C^{\beta}H_2$ × 2), 2.95 (brm, 4H, $^{\omega}$N $C^{\beta}H_2$ × 2), 3.1 (m, 4H, ^{1}N $C^{\beta}H_2$ × 2), 4.32 (H_2), 3.65 (ss, 12H,$COOCH_3$ × 4), 4.35 (m, 4H, ^{1}N $C^{\alpha}H$ × 2 + $^{\omega}$N $C^{\alpha}H$ × 2), 4.55 (q, 2H, pep $C^{\alpha}H$ × 2), 5.05 (s, 8H, Z CH_2 × 2), 7.3 (s, 20H, aromatic), 7.6 (d, 2H, $^{\omega}$NH × 2), 7.9 (d, 2H, ^{1}NH × 2), 8.6 (d, 2H, pep NH × 2)

The bis peptides represented by (96) and (97) constitute novel and unusual structures, wherein within a 22 atom frame work are inscribed 3 disulphide bonds and two peptide linkages and provide opportunities for the formation of secondary structures. A knowledge pertaining to this was considered essential to assess the alignment of three pairs of disulphide linkages.

A major directing force with respect to the overall structure of (96) and (97) would

be the dihedral angle around disulphide bond, generally observed around 90°. Using this as the basis two structural models for these compounds could be constructed as shown in CHART C.III.6 and CHART C.III.7b.

CHART C.III.6 shows that keeping the disulphide dihedral angle around 90° and intramolecular hydrogen bonding involving the peptides, would lead to a helical structure. Such a helical structure would have to be stabilised by hydrogen bonding with the end groups. On the other hand, intermolecular hydrogen bonding of the peptide bonds, while keeping the dihedral angle of the disulphide around 90 degrees, a structural profile as shown in CHART C.III.7a would lead to novel β-sheet representation. The UV spectrum of (96) and (97) was not very revealing because of masking by intense aromatic absorption around 250 nm. The CD spectrum taken in CH_3CN showed a pronounced positive effect around 220 nm and trough around 203 nm. Thus the CD spectra profile appear to exclude α-helical structures leaving the option open either for non-random or sheet type structures.

A wealth of information was secured by detailed NMR studies involving both (96) and (97). These tend to strongly support a novel sheet type representation as shown in CHART C.III.7a in non-polar solvents like chloroform and an open structure as shown in CHART C.III.7b in DMSO. The results of the proton decoupling experiments in $CDCl_3$ of (96) are presented in SECTION.D. They have enabled the identification of the three different sets of C^{α}-protons. In addition decoupling of Pep-$C^{\alpha}H$ has enabled the identification of the position of Pep-$C^{\beta}H_2$.

Difference NOE experiments in DMSO-d_6 (SECTION.D) has provided significant information pertaining to the spatial proximity of several key bonds. Irradiation of the Pep-NH resulted in strong NOE connections with its own $C^{\alpha}H$ as well as with Z-$C^{\alpha}H$. Weak connections were seen with Boc-NH and Z-$C^{\beta}H$. Irradiation of Z-NH did not exhibit any connection. Irradiation of Boc-NH resulted in modest NOE connection with

CHART C.III.6

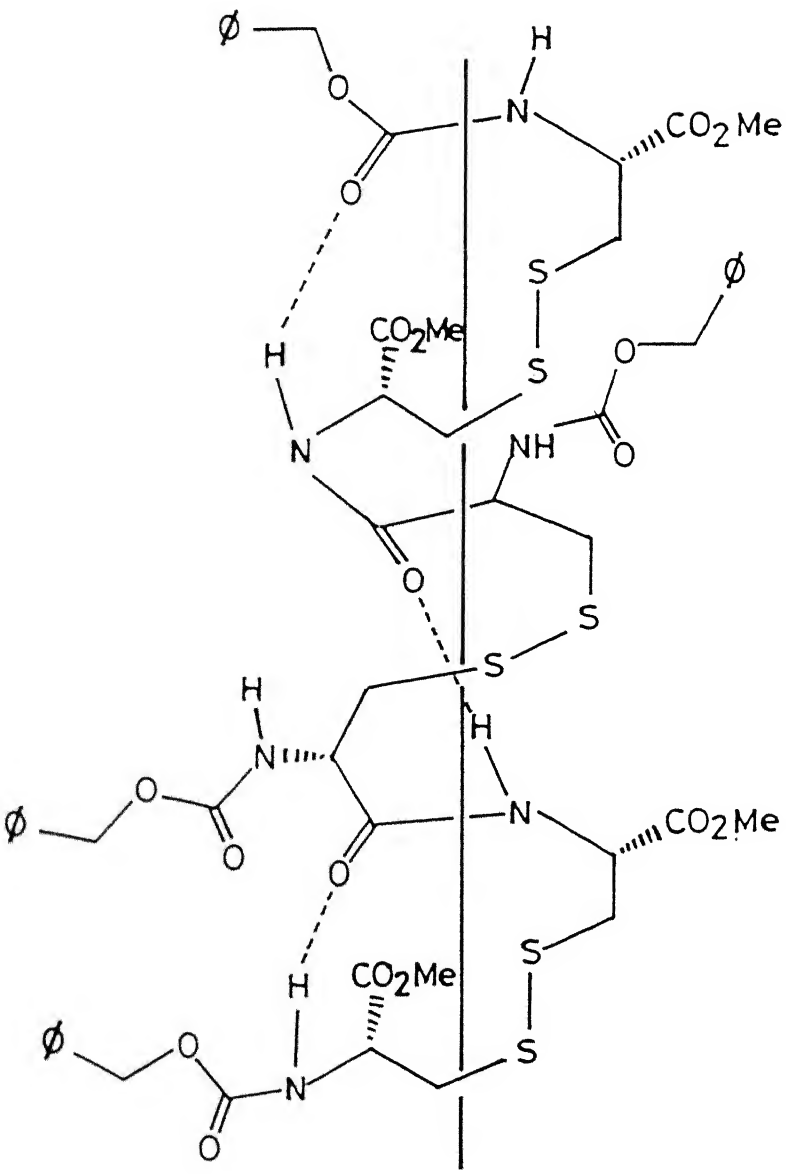

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CHART C.III.7a

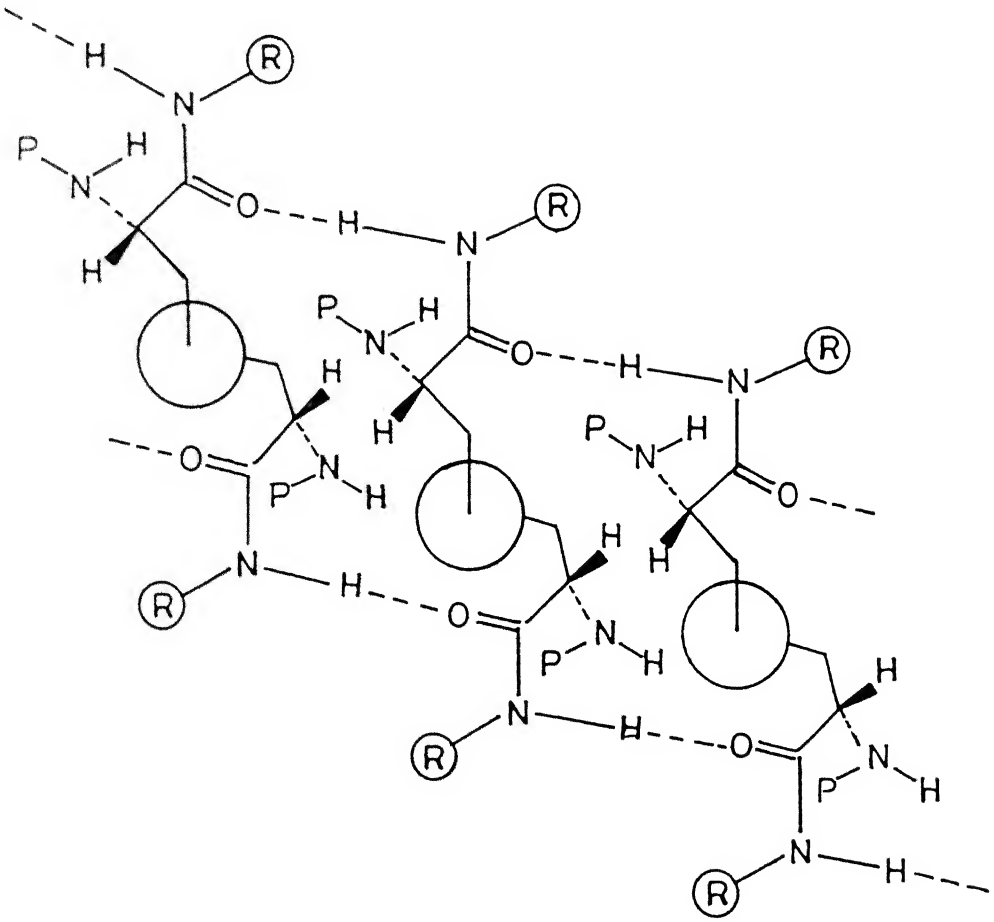
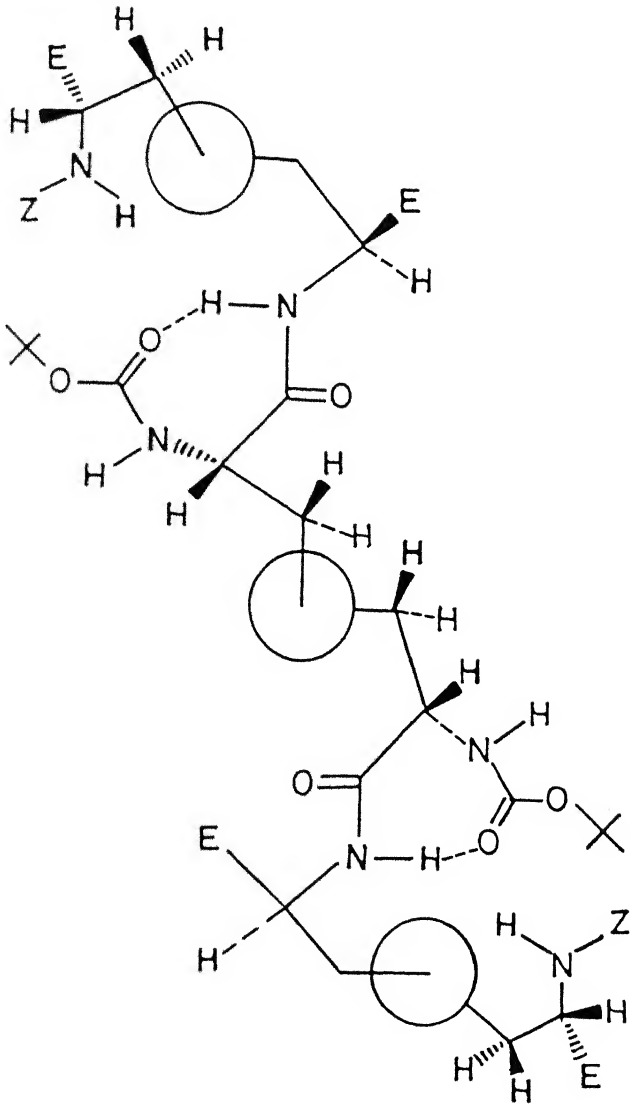


CHART C.III.7b



Pep-NH and rather strong connection with its own $C^{\alpha}H$ as well as Pep-$C^{\alpha}H$ and Z-$C^{\alpha}H$. Modest NOE connection was also observed with Boc-$C^{\beta}H$. These experiments clearly show that unlike the NH located at the terminal positions both Pep-NH and Boc-NH are in close proximity. When coupled with the fact that the latter two are in vicinity of all the three $C^{\alpha}H$ linkages, the conformation of (96) in DMSO should have a profile similar to that shown in CHART C.III.7b.

The NOESY spectrum of (96) in $CDCl_3$ (SECTION.D) reveal connectivity between Z-$C^{\alpha}H$ $\longrightarrow$ Z-$C^{\alpha}H$, Z-$C^{\alpha}H$ $\longrightarrow$ Z-NH and Pep-$C^{\alpha}H$ $\longrightarrow$ Pep-NH.

Solvent titration studies and variable temperature (VT) studies were complementary. Solvent shift measurements with (96) as a function of increasing amount of DMSO-d_6 (SECTION.D) are tabulated in TABLE C.III.1. As could be seen from TABLE C.III.1, the shift of 2.06 ppm for the Z-NH is noteworthy and clearly show that it is normally solvent exposed. An intermediate value of 1.43 ppm for the Boc-NH indicates it is involved in weak association. The very low value of 0.58 ppm shift for the Pep-NH tend to show that it is intramolecularly hydrogen bonded in DMSO.

Variable temperature studies with (96) in $CDCl_3$ were found to be quite reliable with respect to the Pep-NH and showed a $d\delta/dT$ value of -7.1 ppb/K clearly showing that the Pep-NH are not involved in intramolecular hydrogen bonding in this solvent. With respect to Z-NH and Boc-NH, in $CDCl_3$, $d\delta/dT$ values respectively -4.5 ppb/K and -1.7 ppb/K were obtained.

The rather low value for the Boc-NH in $CDCl_3$ (-1.7 ppb/K) compared to a value of -5.8 ppb/K in DMSO clearly show that Boc-NH is not involved in intramolecular hydrogen bonding. The situation with respect to Z-NH is not very clear. It showed large negative values both in $CHCl_3$ (-4.5 ppb/K) and in DMSO (-6.6 ppb/K). These coupled with the very large solvent shift of -2.06 ppm tend to suggest that this NH could

TABLE C.III.1 NH Shift from solvent titration ($CDCl_3$-DMSO-d_6) studies on(96)

% DMSO-d_6	0	2	4	6	8	10	16	20	40	100	Σ
PEP NH	7.8625	7.8750	7.8875	7.9012	7.9375	7.9687	8.0437	8.075	8.1875	8.45	0.58
Z NH	5.8375	6.0625	6.2375	6.4562	6.600	6.7750	7.1125	7.2375	7.5375	7.90	2.06
Boc NH	5.6187	7.7125	5.800	5.9125	5.9875	6.1000	6.3250	6.4187	6.6750	7.05	1.43

be solvent exposed in $CHCl_3$. The shift in $CDCl_3$ could also be explained in terms of random disposition of terminal positions in (96) thus offering possibilities for thermal equilibration.

Variable temperature NMR studies of (96) in DMSO were very clean (SECTION.D). In this solvent $d\delta/dT$ values of -3.9 ppb/K, -6.6 ppb/K and -5.8 ppb/K were obtained respectively for Pep-NH, Z-NH and Boc-NH showing that they are hydrogen bonded to the solvent. As stated previously very low value of 0.58 ppm in solvent titration studies for the Pep-NH coupled with $d\delta/dT$ values in $CDCl_3$ and DMSO-d_6 supports strongly that the Pep-NH bonds are associated in non-polar solvents and are intramolecularly hydrogen bonded in DMSO. All the studies thus far shown supports a structural profile shown in CHART C.III.7a for (96) in non-polar solvents and a structure shown in CHART C.III.7b in DMSO.

The notion that peptide bonds in (96) are associated in non-polar solvents is confirmed by similar studies with (97).

An interesting finding was that whereas the Z-NH protons in (97) could be readily exchanged with D_2O as could be seen in NMR spectra in $CDCl_3$, the Pep-NH was not affected. Proton decoupling studies (SECTION.D) in $CDCl_3$ enabled the correlation of all the NH units with their $C^{\alpha}H$. In addition irradiation of Pep-$C^{\alpha}H$ led to identification of its own $C^{\beta}H$.

Difference NOE studies in DMSO-d_6 (SECTION.D) showed spatial connectivity of Pep-NH with its own $C^{\alpha}H$ and ^{1}N-$C^{\alpha}H$. Weak NOE connections were also seen with $^{\omega}$NH. Irradiation of ^{1}NH showed weak connections with its own $C^{\alpha}H$.

The results of solvent titration experiments (SECTION.D) are tabulated in TABLE C.III.2. The large solvent shift observed (2.07 ppm) with ^{1}NH is very similar to that of (96) showing that this proton is highly solvent exposed. An intermediate value of

TABLE C.III.2 NH Shift from solvent titration ($CDCl_3$-DMSO-d_6) studies on(97)

% DMSO-d_6	0	2	4	6	8	10	16	20	40	100	Σ
PEP NH	8.1937	8.1687	8.1562	8.1652	8.1652	8.1687	8.1812	8.1937	8.2750	8.6	0.41
^{1}NH	5.8375	6.0375	6.2375	6.4437	6.6187	6.7625	6.9625	7.2250	7.4250	7.9	2.07
^{3}NH	5.900	6.100	6.2375	6.4125	6.5687	6.6937	6.8625	7.000	7.2437	7.6	1.7

TABLE C.III.3 : $-\frac{d\delta}{dT}$ values of (96) and (97) in $CDCl_3$ and DMSO-d_6

^1H	(96)		(97)	
	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6
Pep NH	7.1	3.9	6.2	6.06
Z NH	4.5	6.6	5.3	6.9
Boc NH	1.7	5.8	-	-
Z $^{\omega}$NH	-	-	1.09	6.06

hyde it underwent condensation with cystine-diOMe to afford the Schiff base (98) along with other untraceable products. The Schiff base (98) on reaction with propane dithiol (PDT) followed by complexation with $ZnCl_2$ afforded the zinc complex (99), instantaneously, whose mass spectrum, though complex in nature, exhibited a minor peak at 605 corresponding to $(MH)^+$ of the expected bi-metallic cluster (CHART C.III.8).

---

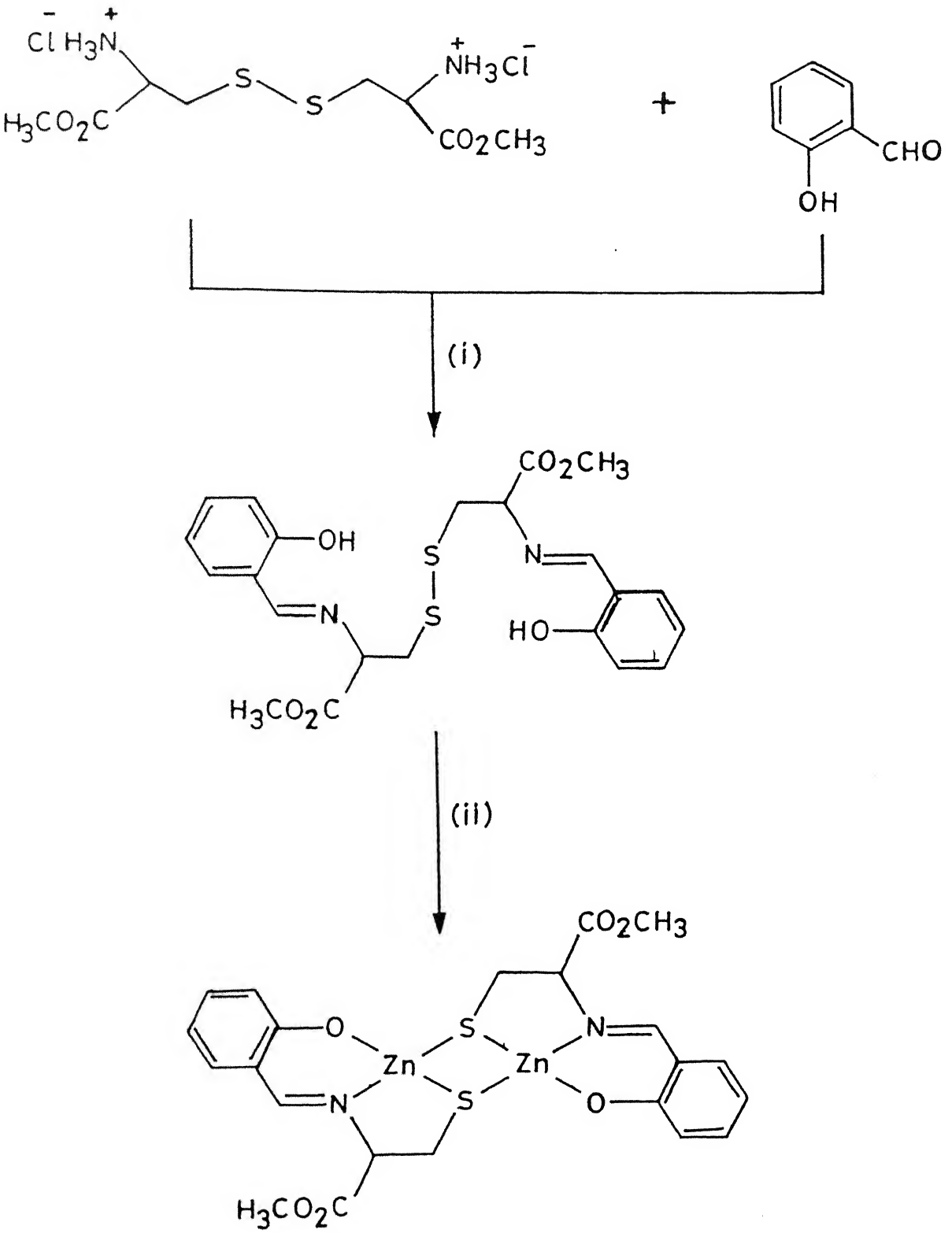
(98)

yield	:	47%
mp	:	gummy
ir(neat)ν_{max} cm^{-1}	:	2954, 1742, 1664, 1624, 1594, 1490, 1458
nmr($CDCl_3$) δ	:	1.47 (d, 4H, cystine $C^{\beta}H_2$), 3.31-4.19 (m, 8H, $COOCH_3$ + cystine $C^{\alpha}H$), 6.66-7.41 (m, 8H, aromatic), 8.31 (s, 2H, -N=CH), 12.16 (s, 2H, OH)

(99)

yield	:	54%
mp	:	>340°C
ir(neat)ν_{max} cm^{-1}	:	3420, 3058, 2925, 1724, 1621, 1548, 1477, 1446
ms (m/z)	:	605 $(MH)^+$

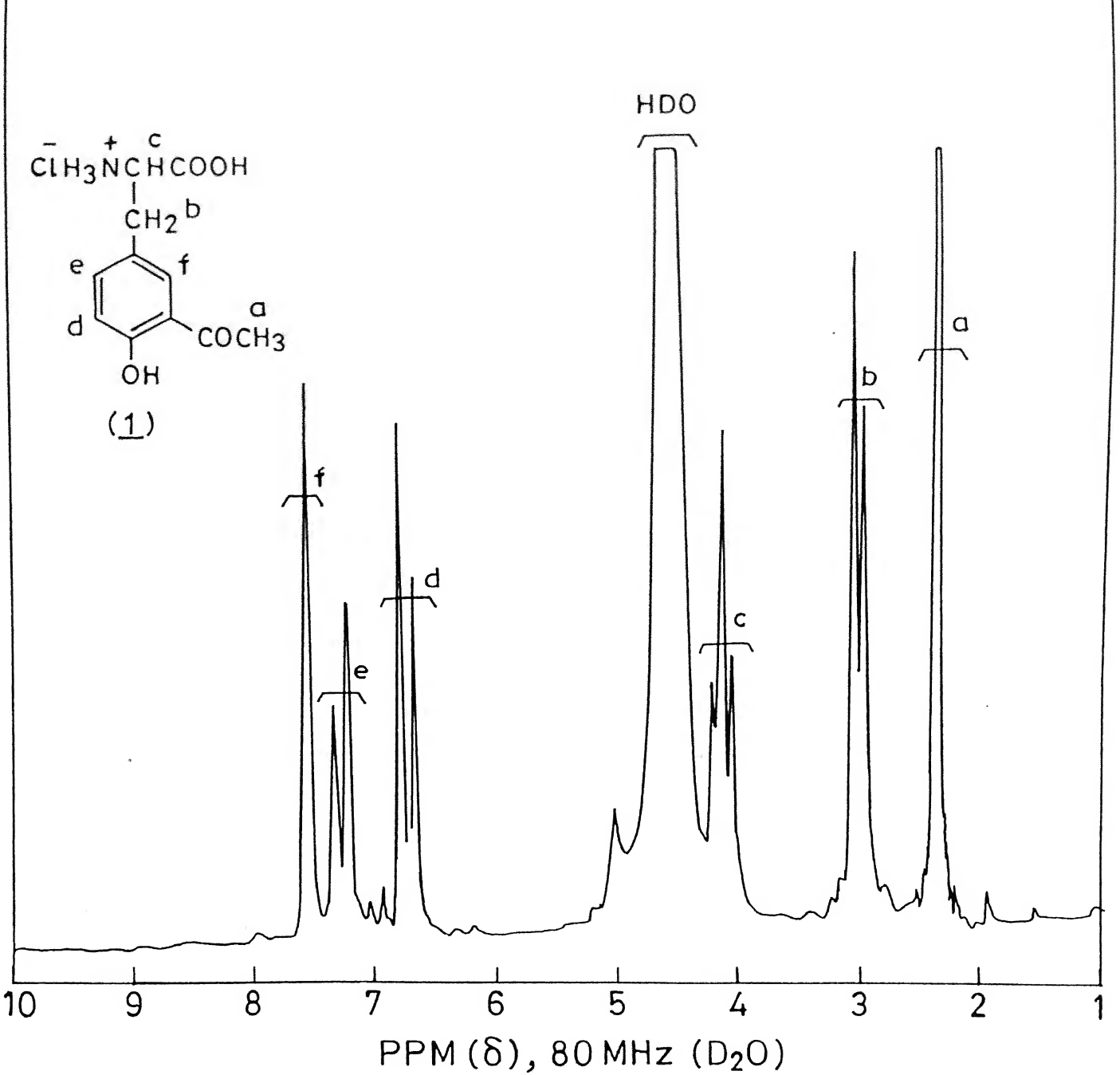
CHART.C.III.8



(i) NEt_3, MeOH, 50°C

(ii) PDT , NEt_3 , $ZnCl_2$, MeOH

D. SPECTRA



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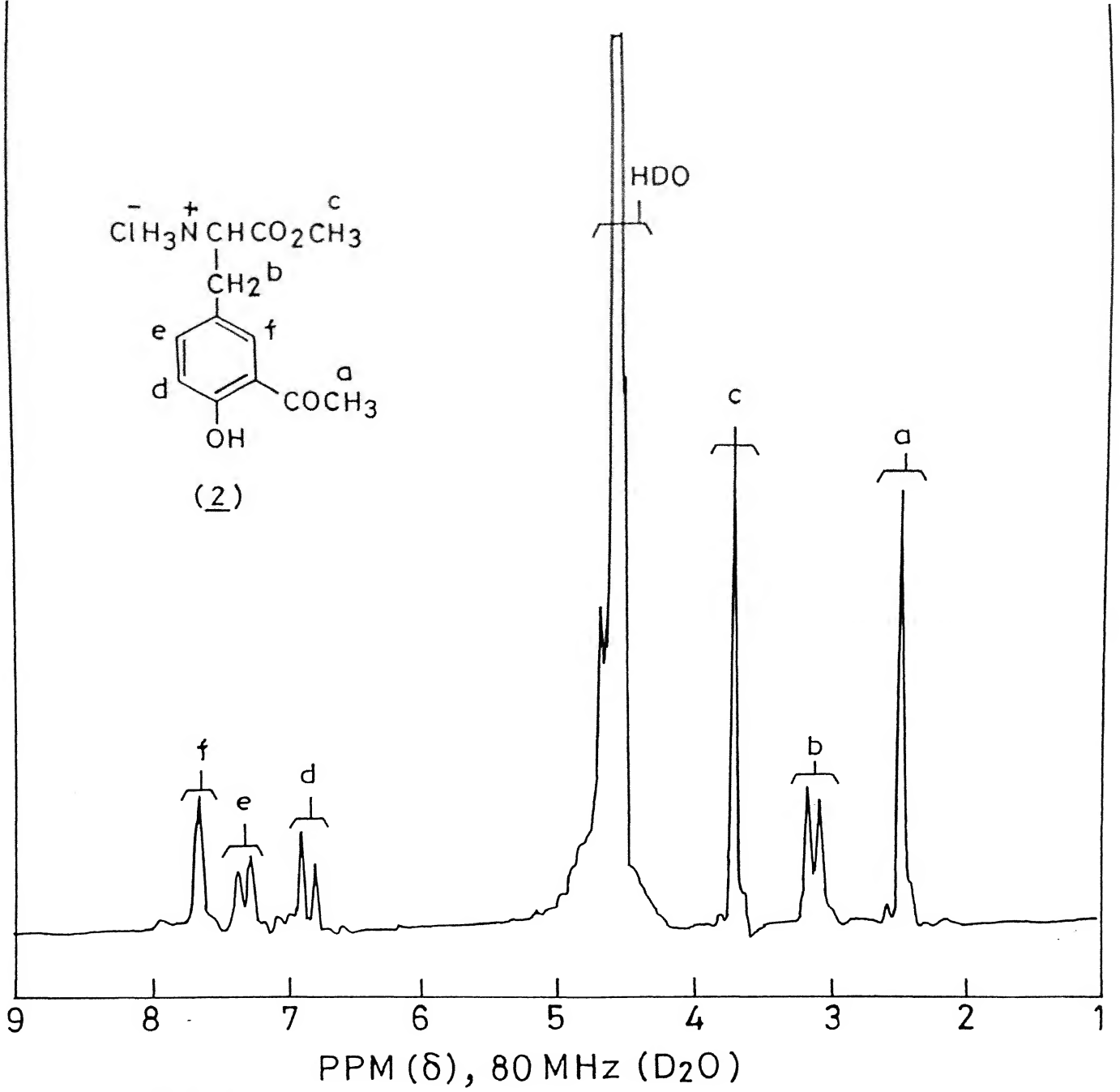

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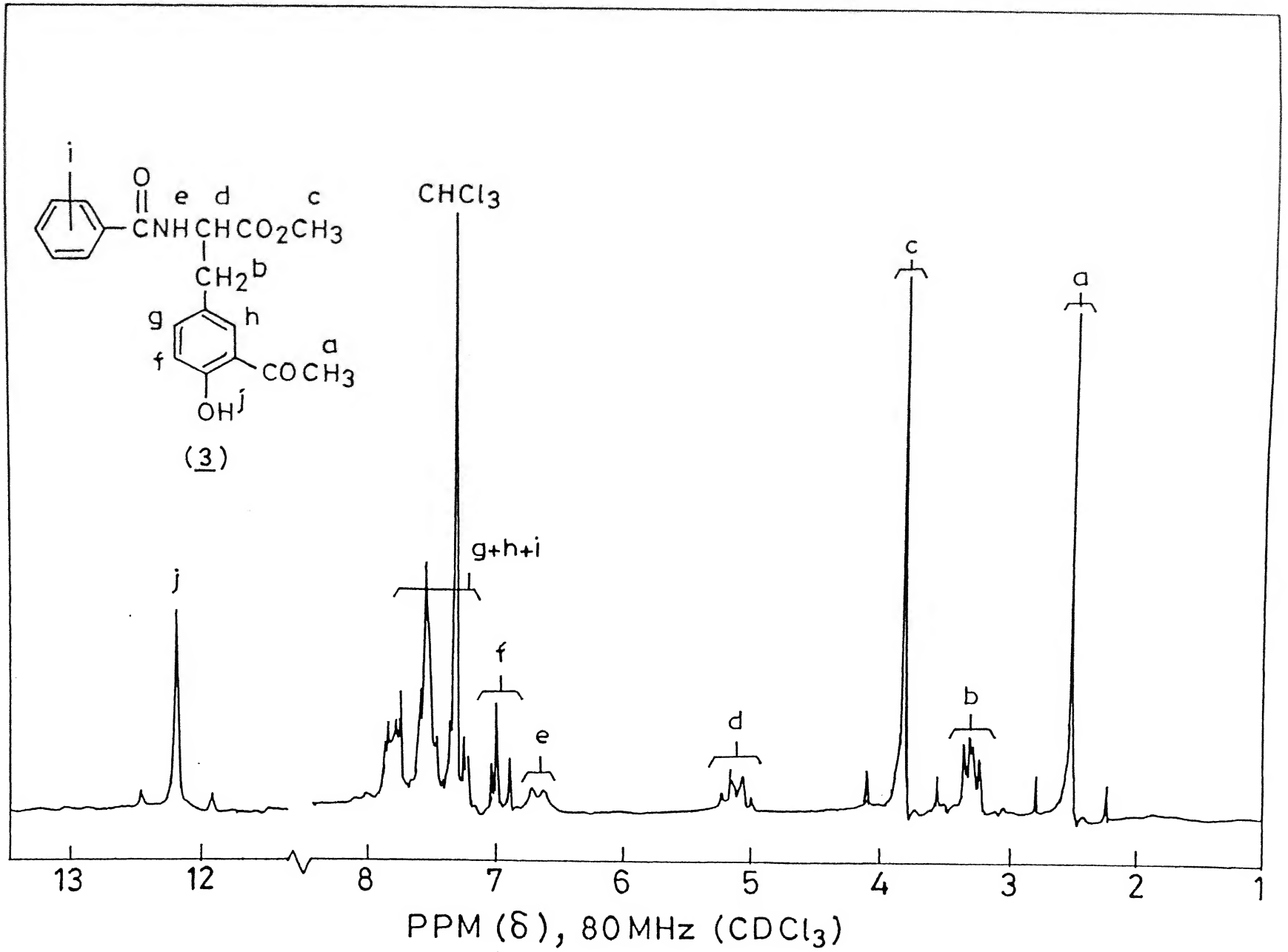
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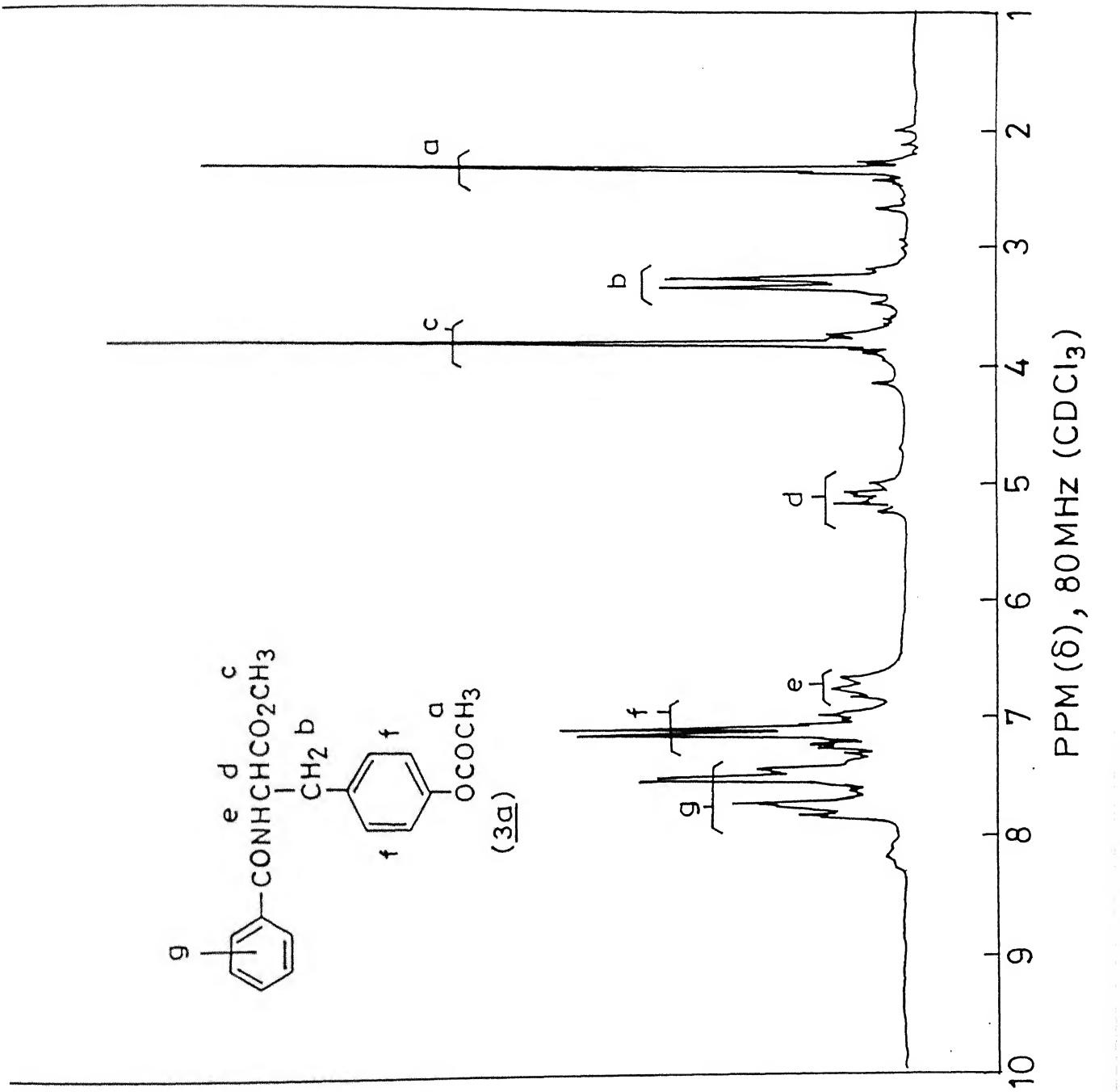
PPM (δ), 80 MHz (CDCl3)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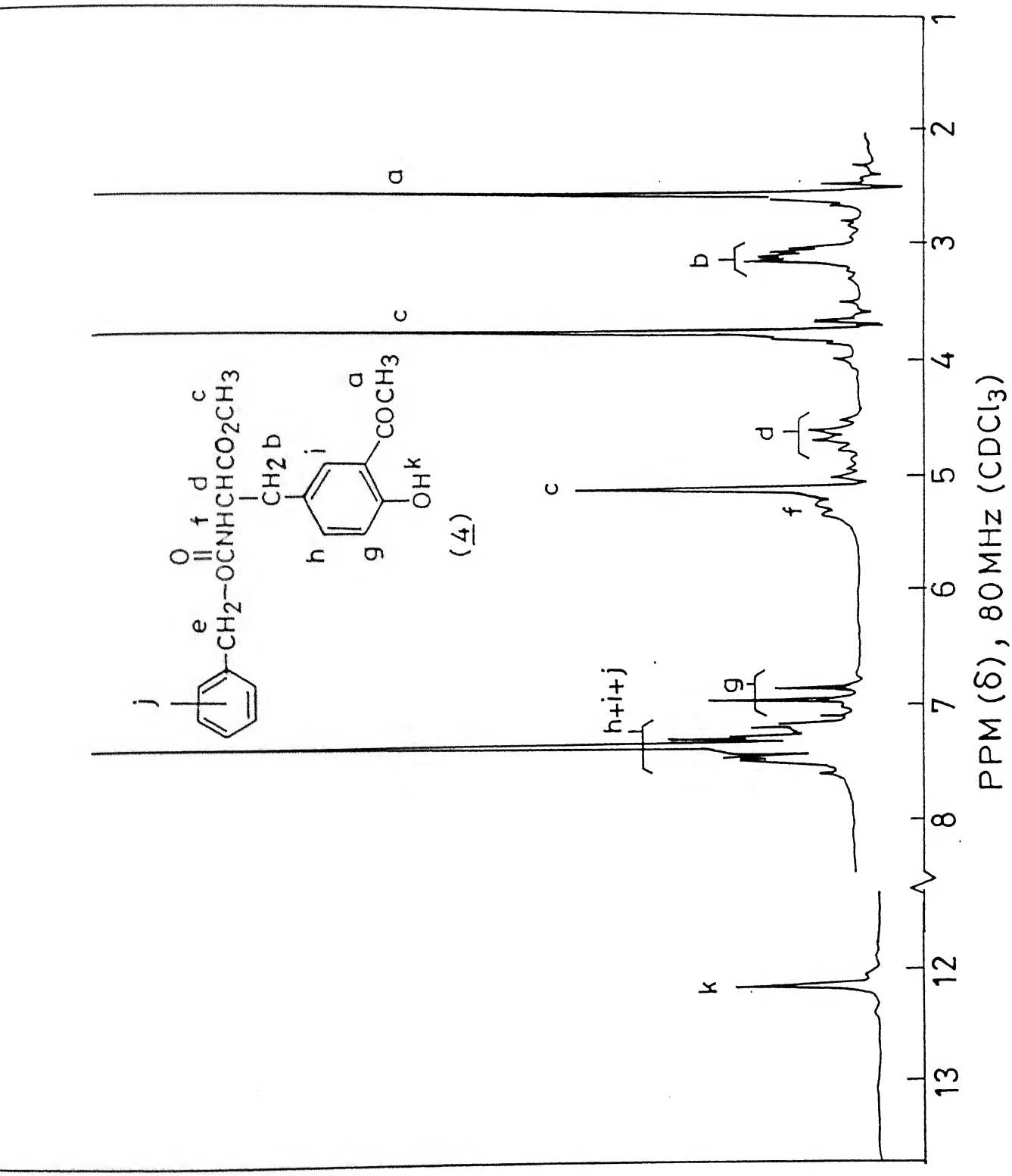
PPM (δ), 80MHz (CDCl3)


PPM (δ), 80 MHz (CDCl3)
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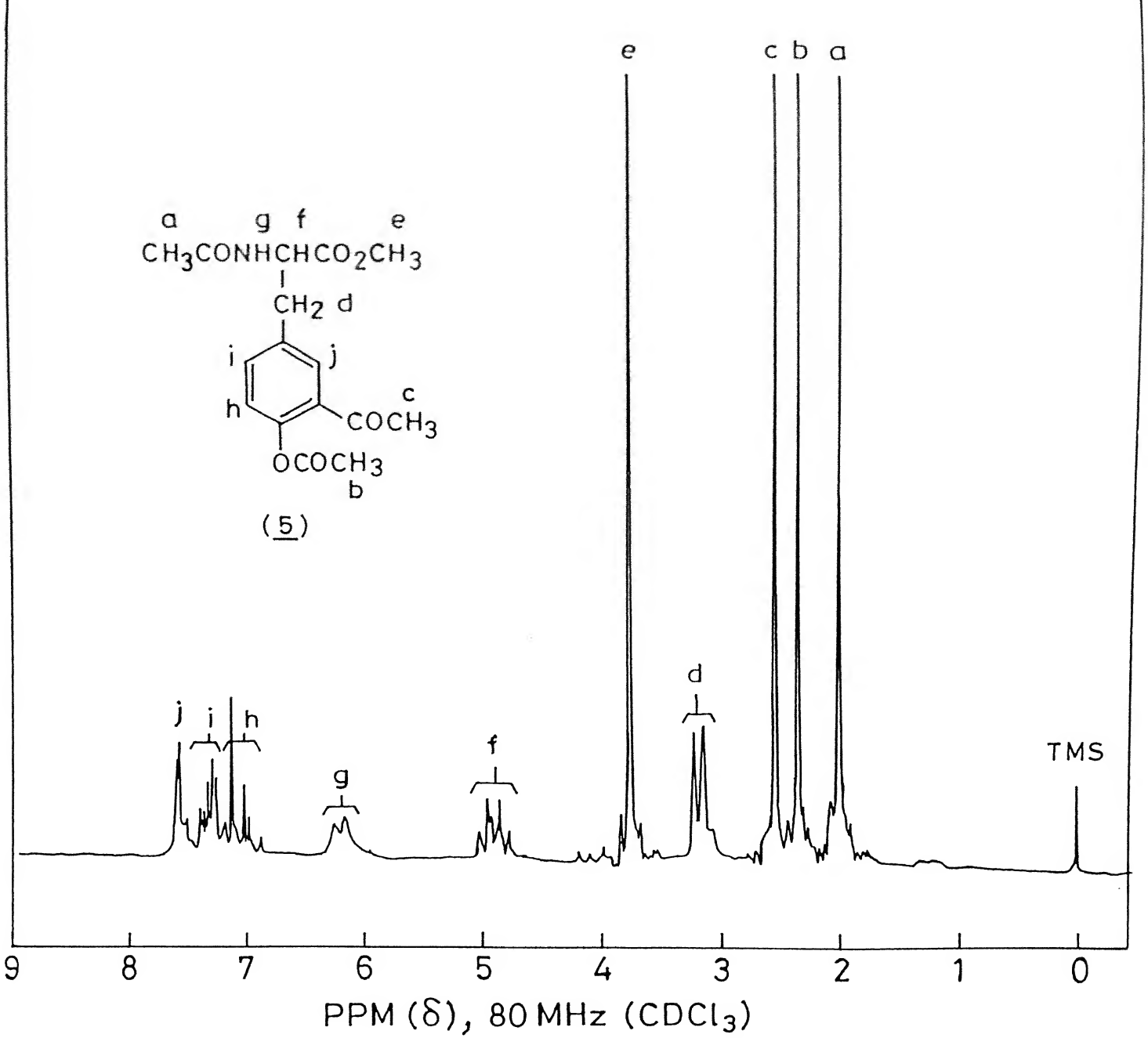
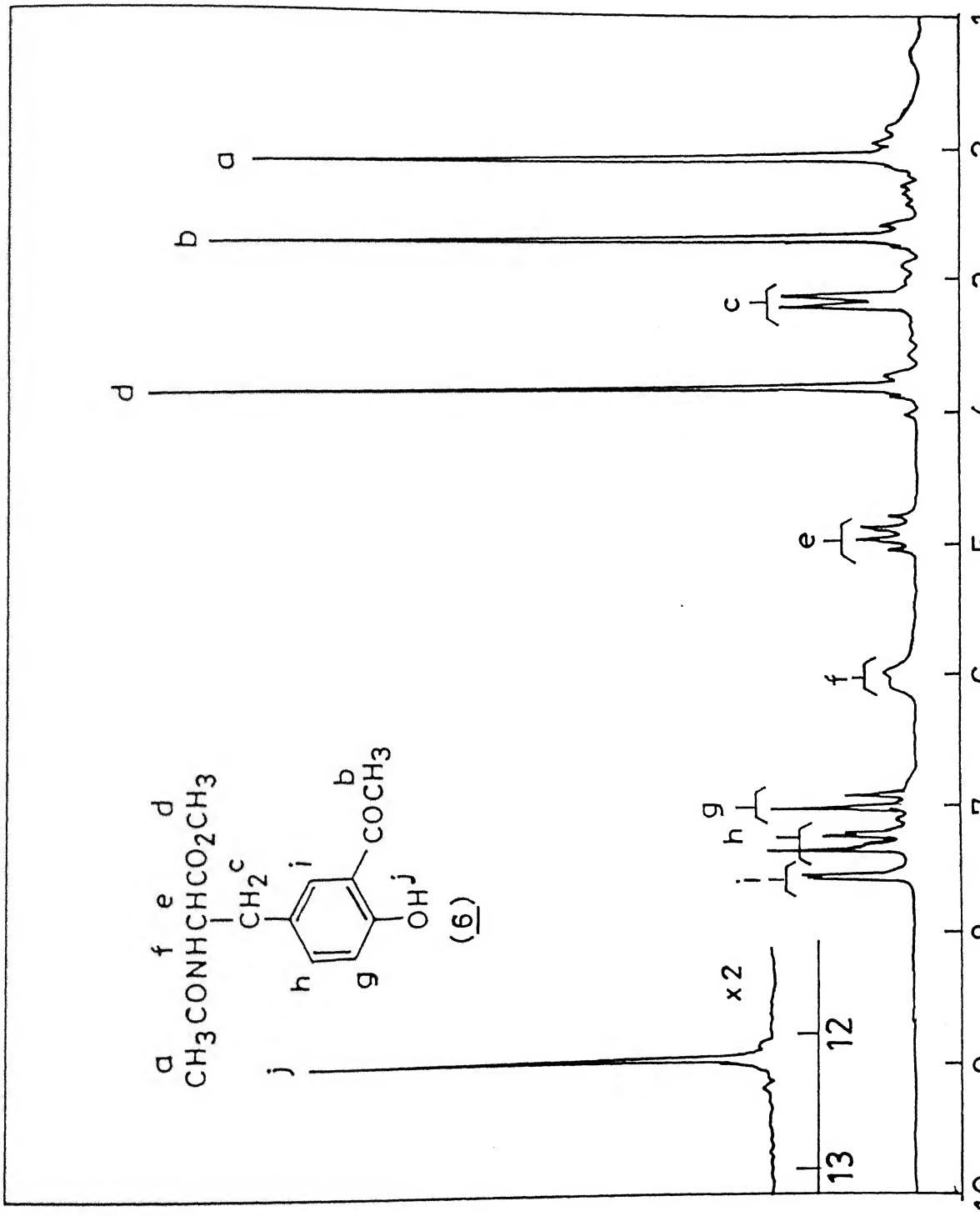
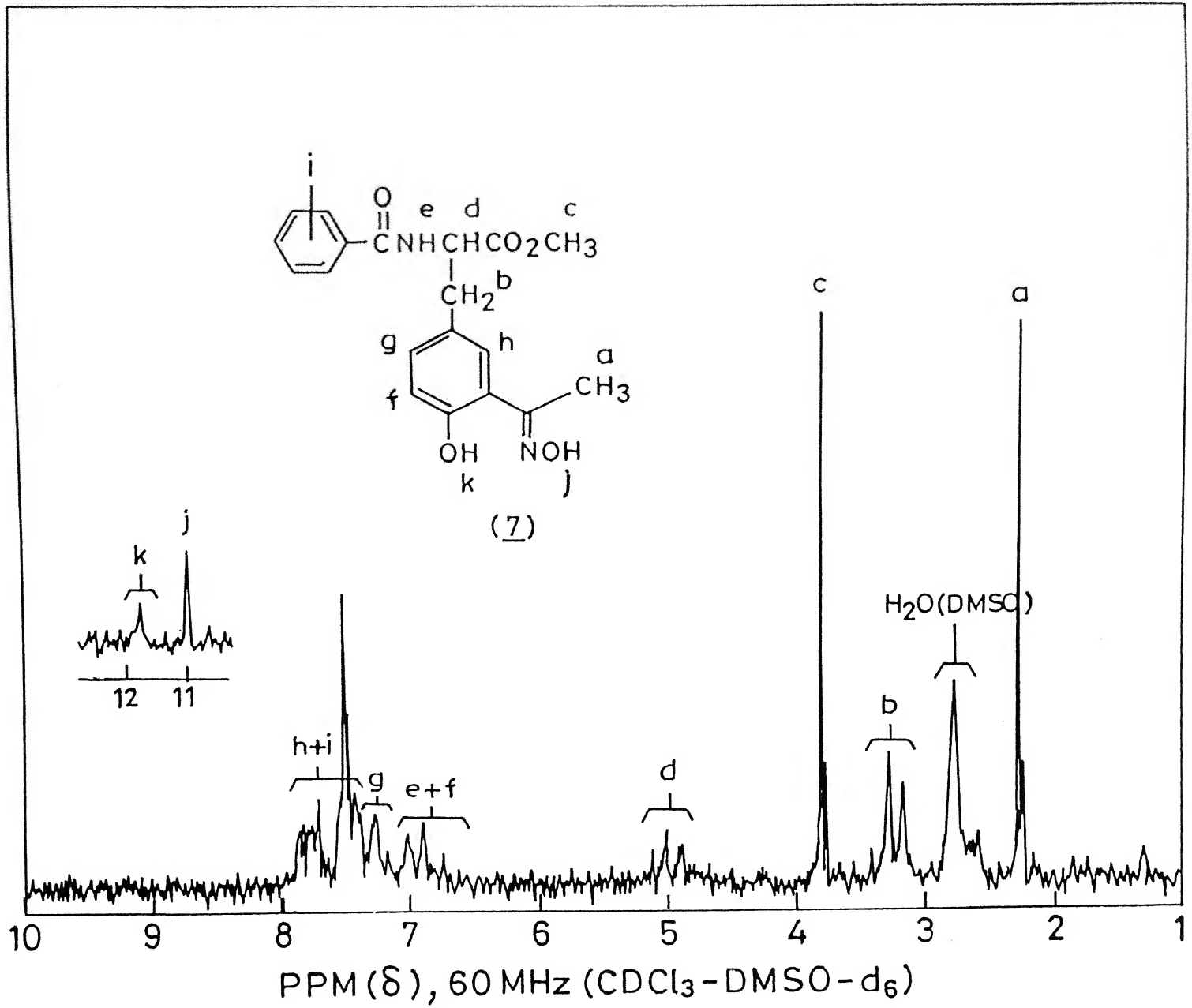
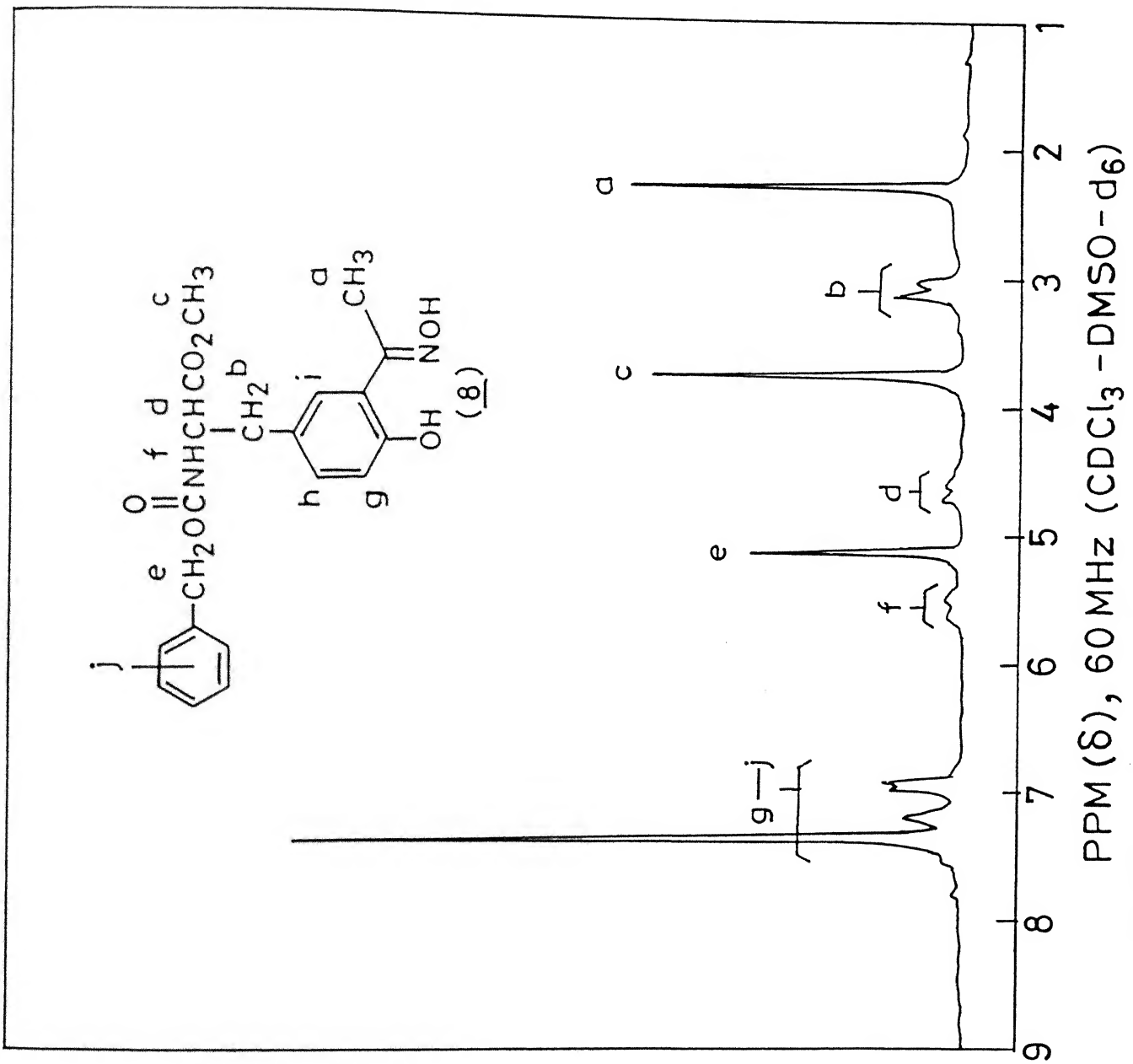
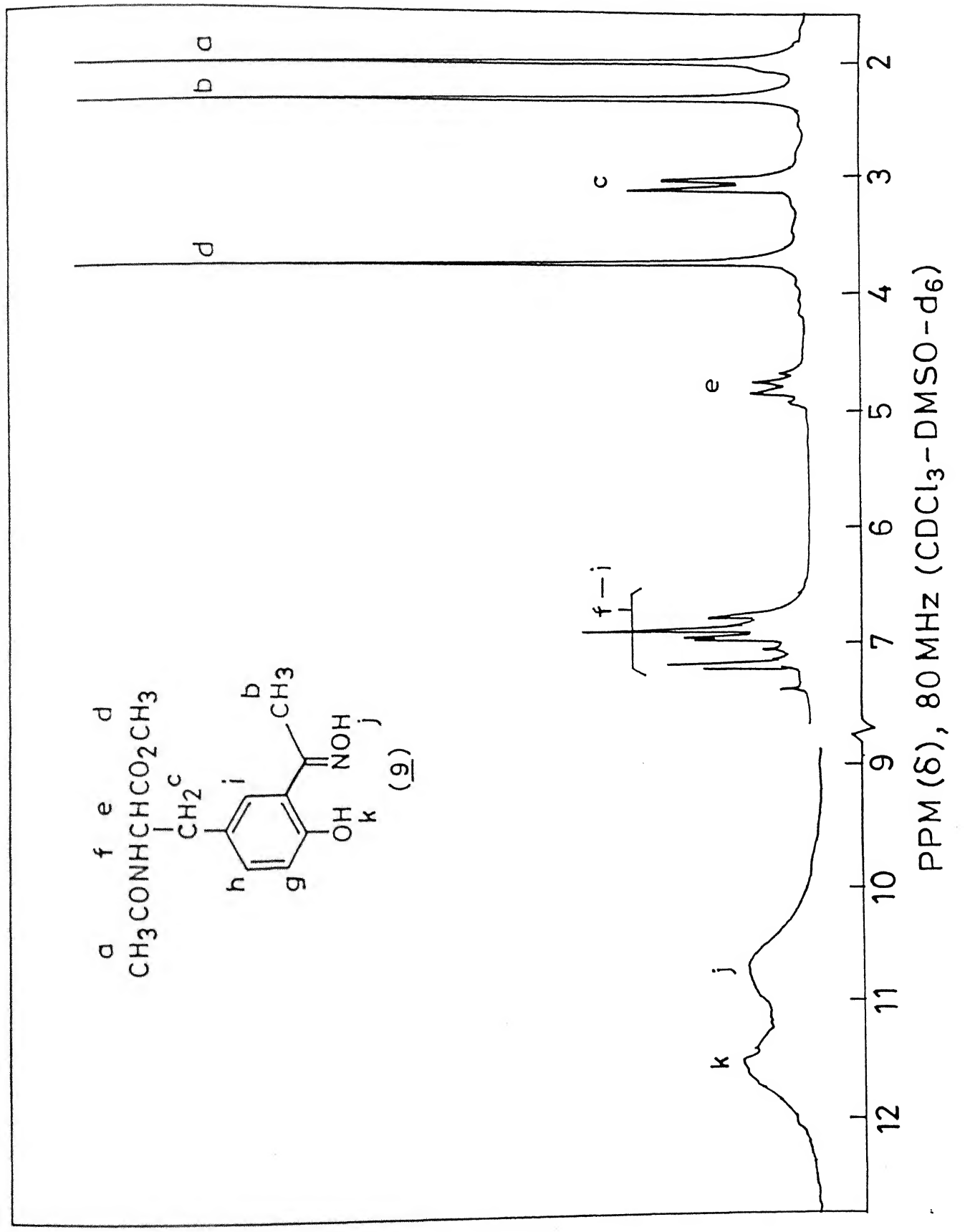
a
(4)

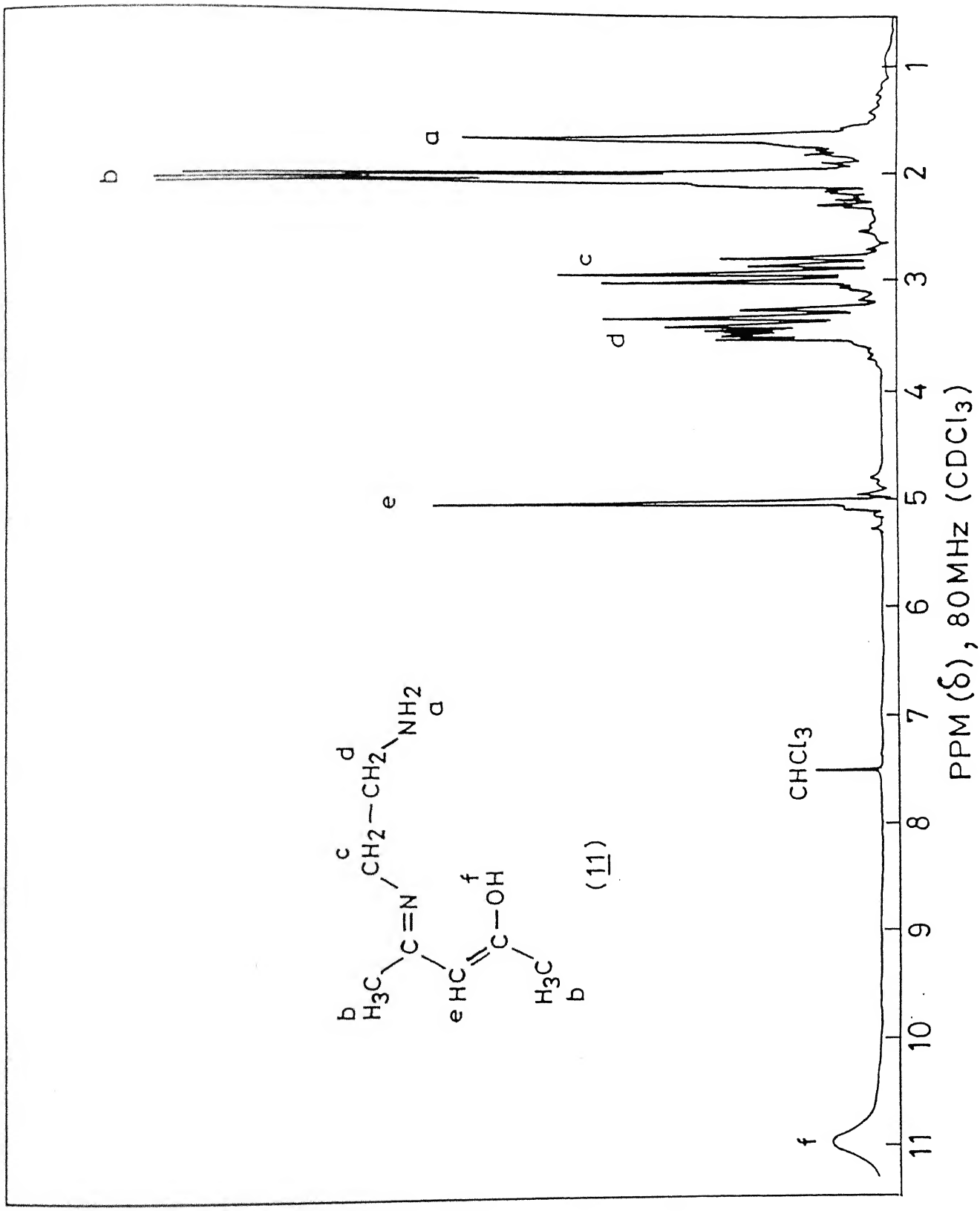

a g f e
CH3CONHCHCO2CH3
CH2 d
i
j
h
c
COCH3
OCOCH3
b
(5)
e
c b a
d
j i h
g
f
TMS
9 8 7 6 5 4 3 2 1 0
PPM (δ), 80 MHz (CDCl3)


a
f e d
CH3CONHCHCO2CH3
CH2 c
i
b
COCH3
h
g
OH j
(6)
j
x2
13
12
i
h g
f
e
d
c
b
a
10 9 8 7 6 5 4 3 2 1
PPM(δ), 80MHz (CDCl3)

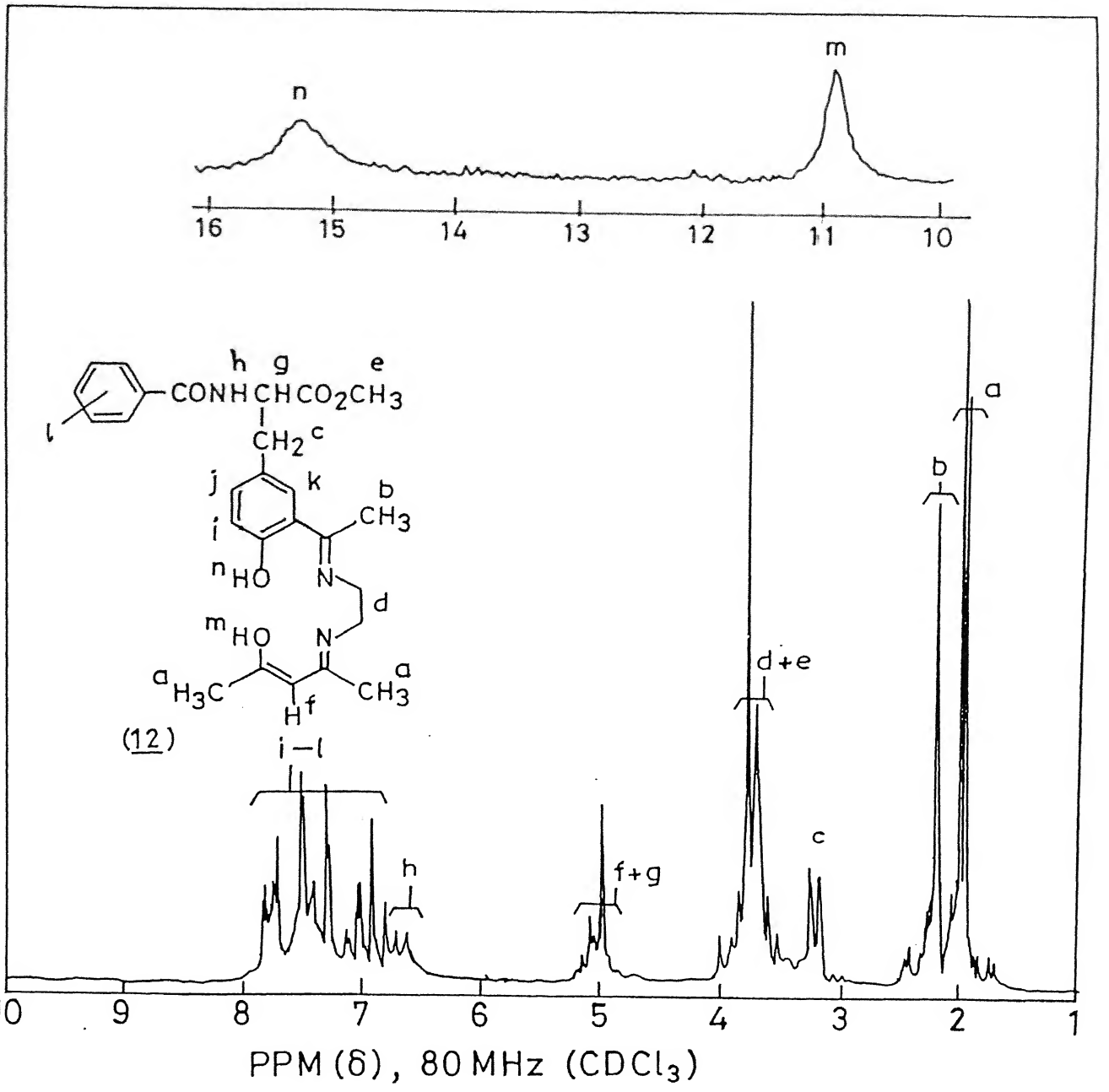

i
O
e d c
CNHCHCO2CH3
CH2 b
g h
a
CH3
f
OH NOH
k j
(7)
k
j
12 11
h+i
g
e+f
d
c
a
b
H2O(DMSO)
10 9 8 7 6 5 4 3 2 1
PPM(δ), 60 MHz (CDCl3-DMSO-d6)

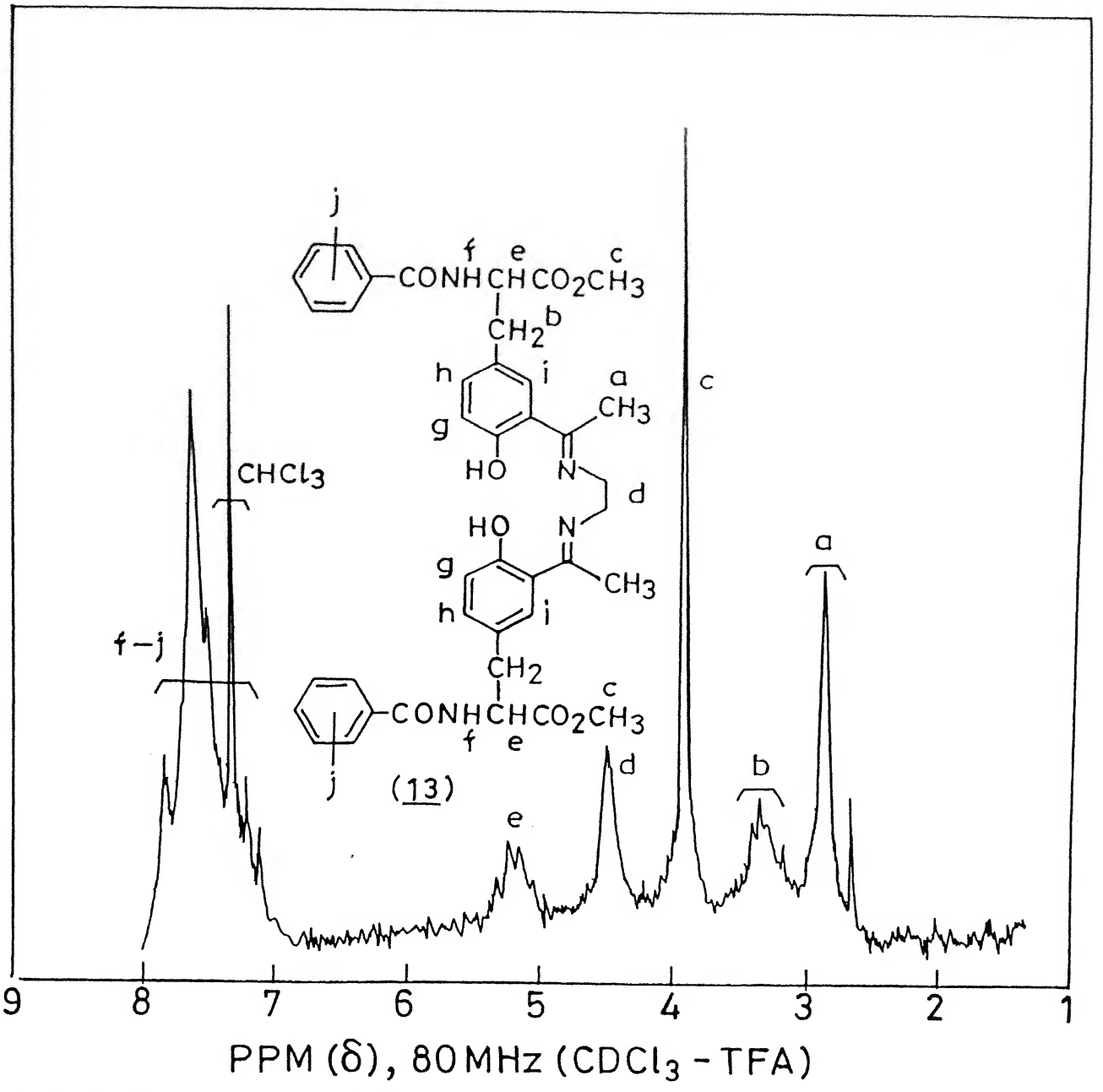

PPM (δ), 60 MHz (CDCl3 - DMSO - d6)
9
8
7
6
5
4
3
2
1
a
b
c
d
e
f
g—j
(8)

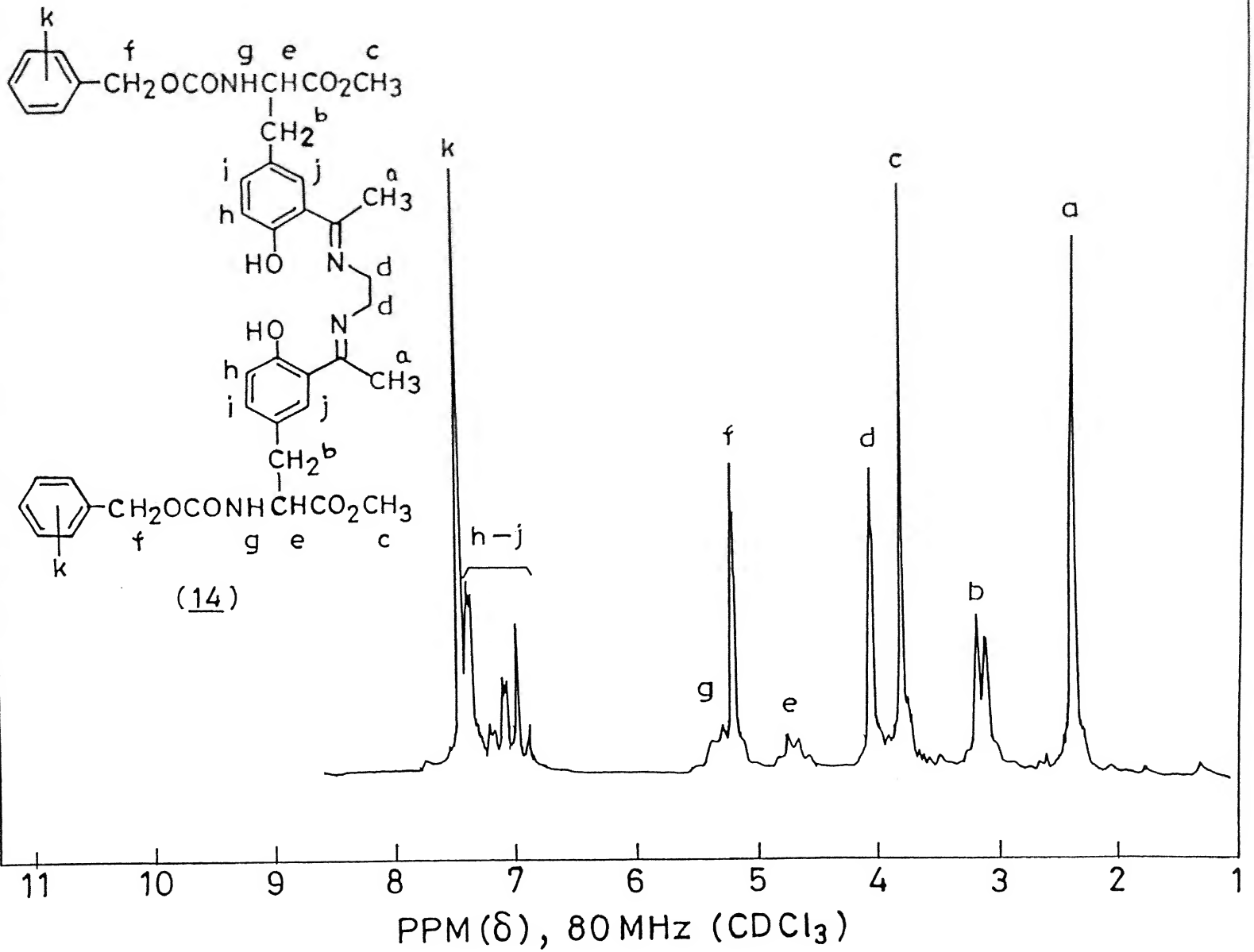

a f e d
CH3CONHCHCO2CH3
CH2
c
i
b
CH3
NOH
j
OH
k
h
g
(9)
a
b
c
d
e
f — i
j
k
2
3
4
5
6
7
9
10
11
12
PPM (δ), 80 MHz (CDCl3–DMSO–d6)

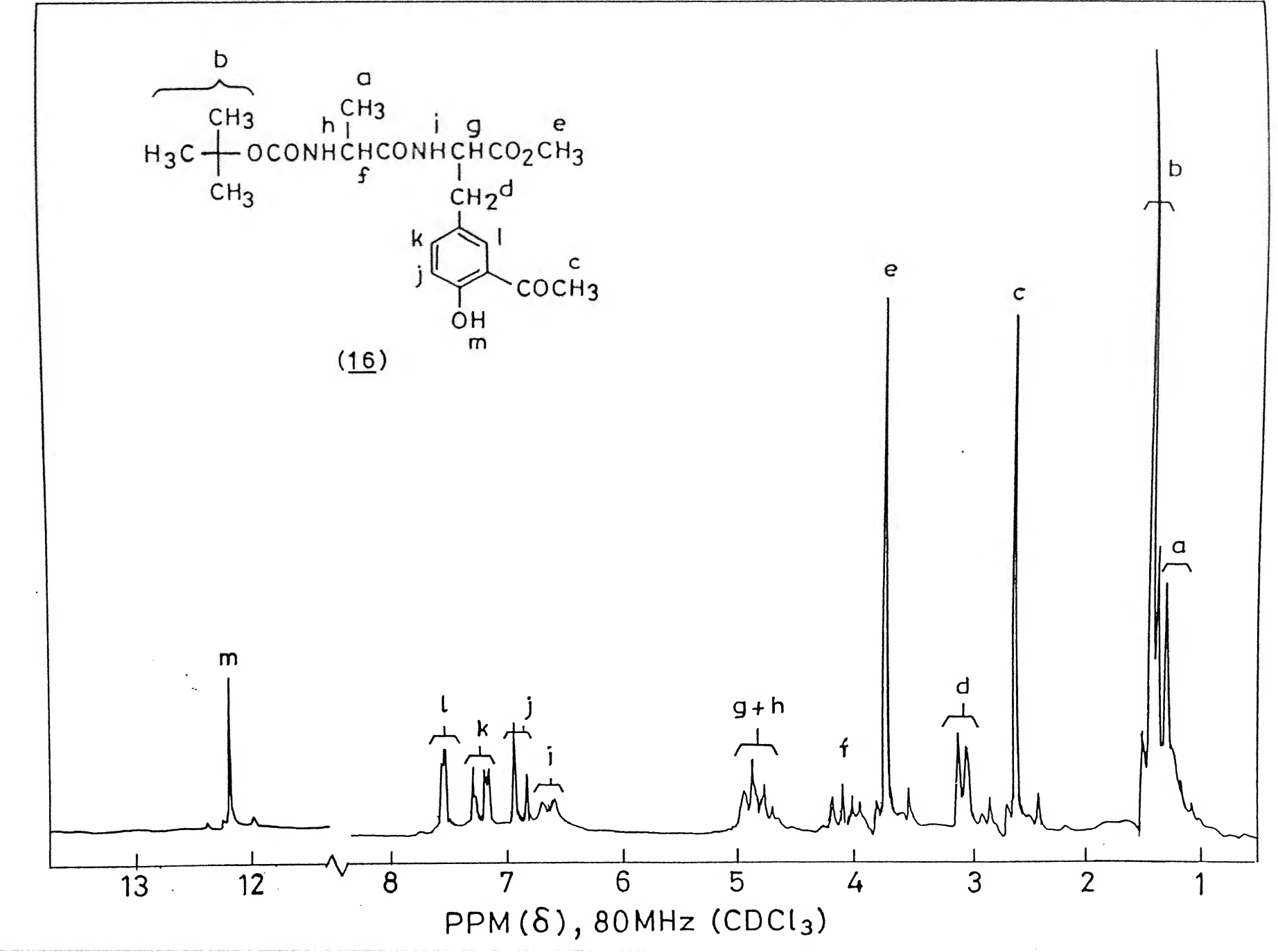

b
a
c
d
e
f
CHCl3
1
2
3
4
5
6
7
8
9
10
11
PPM (δ), 80 MHz (CDCl3)
NH2
CH2
CH2
C=N
HC
C—OH
H3C
H3C

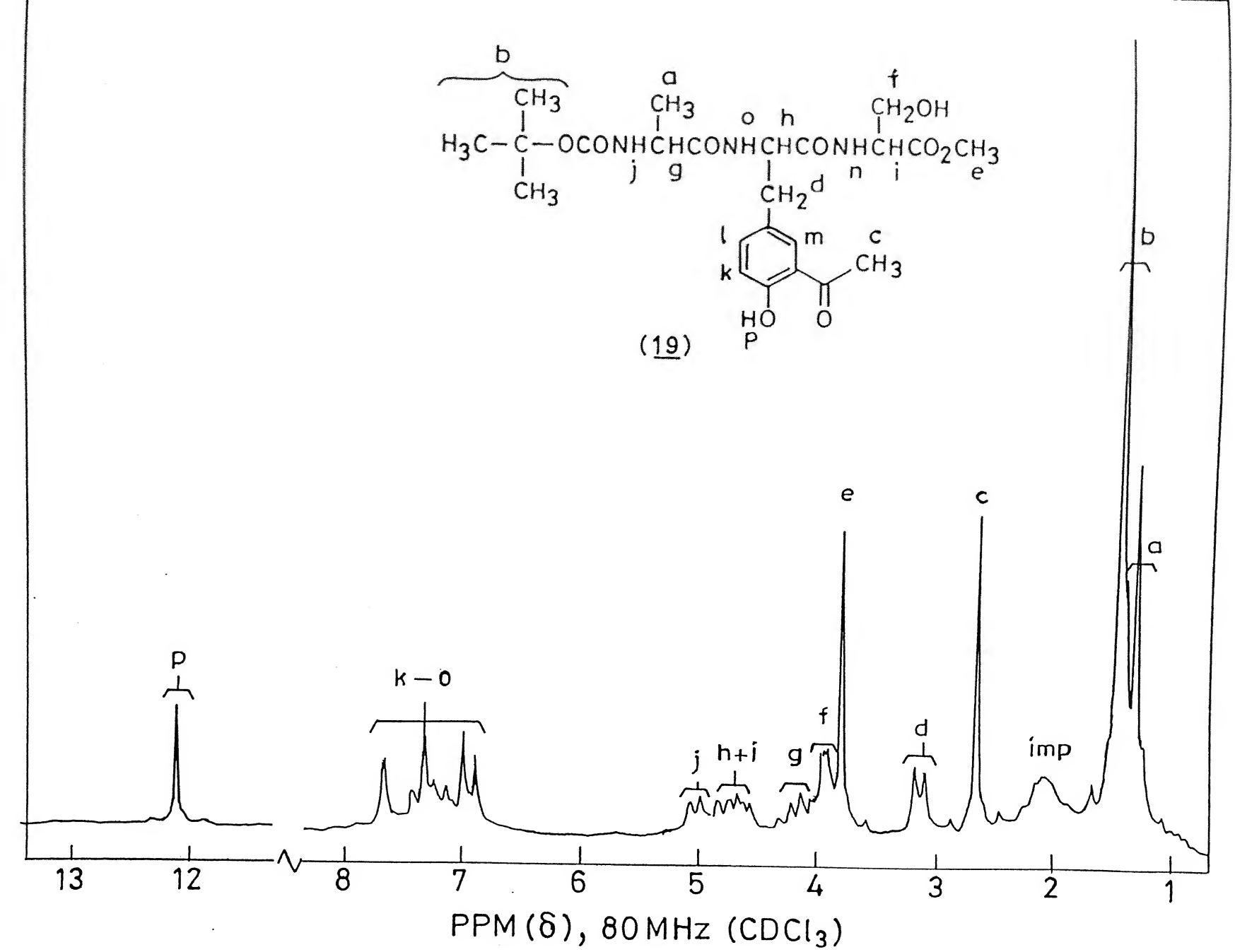
(11)

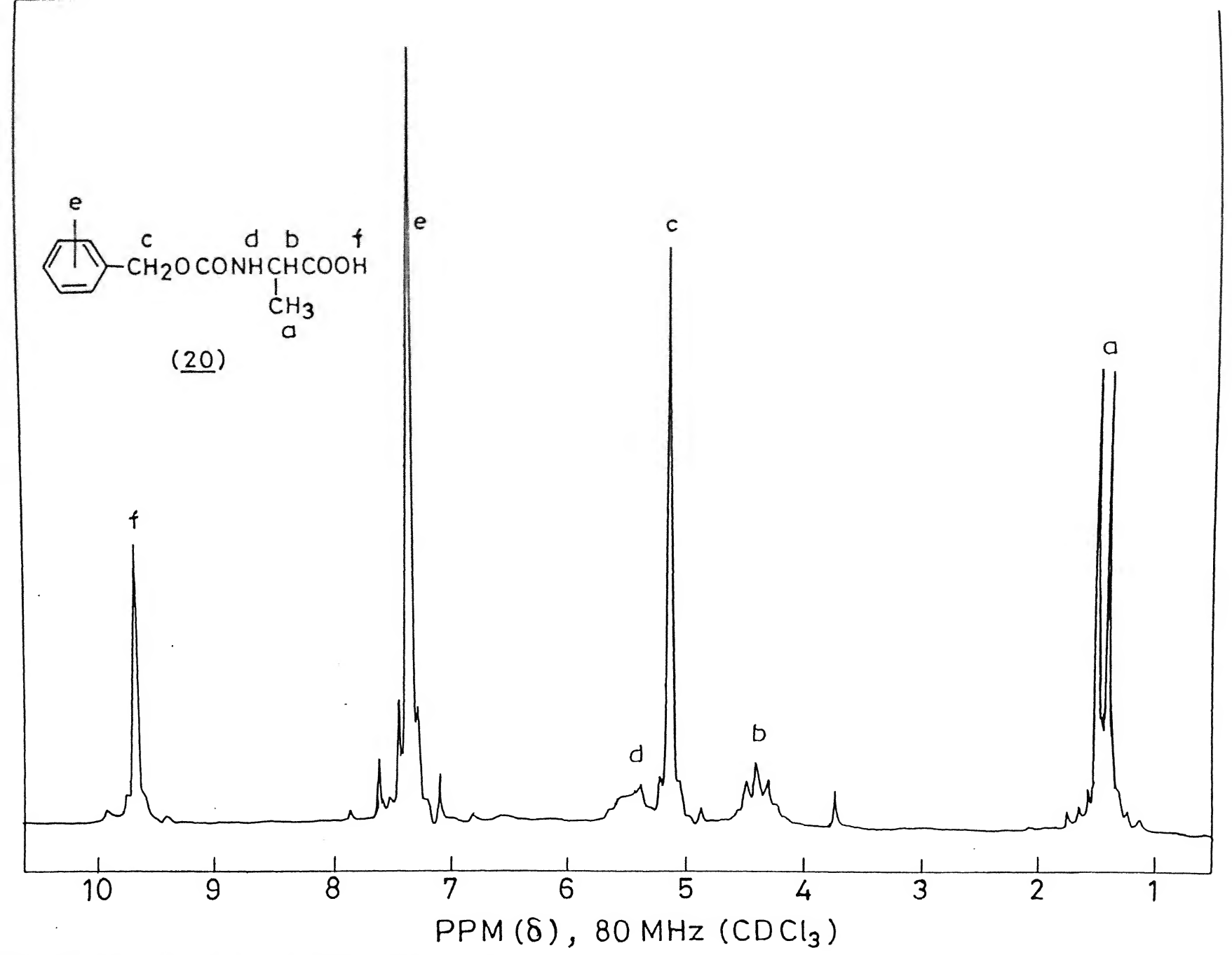

m
n
16
15
14
13
12
11
10
h g e
CONH CH CO2CH3
l
CH2 c
j
k
b
CH3
i
n HO
N
d
m HO
N
a H3C
a
CH3
H f
(12)
i–l
h
f+g
d+e
c
b
a
10
9
8
7
6
5
4
3
2
1
PPM (δ), 80 MHz (CDCl3)

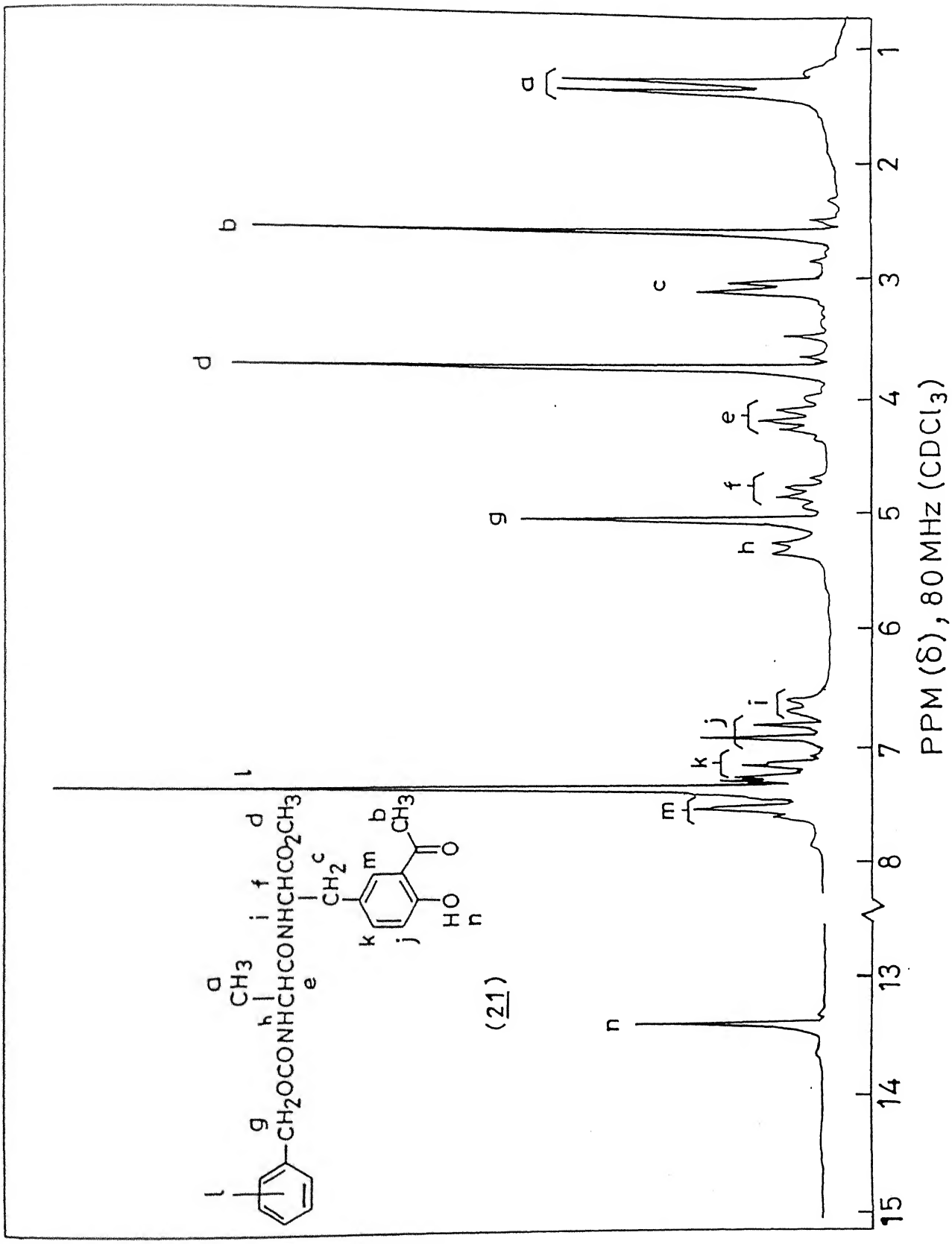

j
f e c
CONHCHCO2CH3
CH2 b
h i a
g CH3
HO N
d
HO N
g CH3
h i
CH2
c
CONHCHCO2CH3
f e
j
(13)
CHCl3
f–j
a
b
d
e
c
9 8 7 6 5 4 3 2 1
PPM (δ), 80 MHz (CDCl3 - TFA)


k
f g e c
CH2OCONHCHCO2CH3
CH2 b
i j
h
a
CH3
HO N
d
d
HO N
h
a
CH3
i j
CH2 b
CH2OCONHCHCO2CH3
f g e c
k
(14)
k
h–j
g
f
e
d
c
b
a
11 10 9 8 7 6 5 4 3 2 1
PPM(δ), 80 MHz (CDCl3)

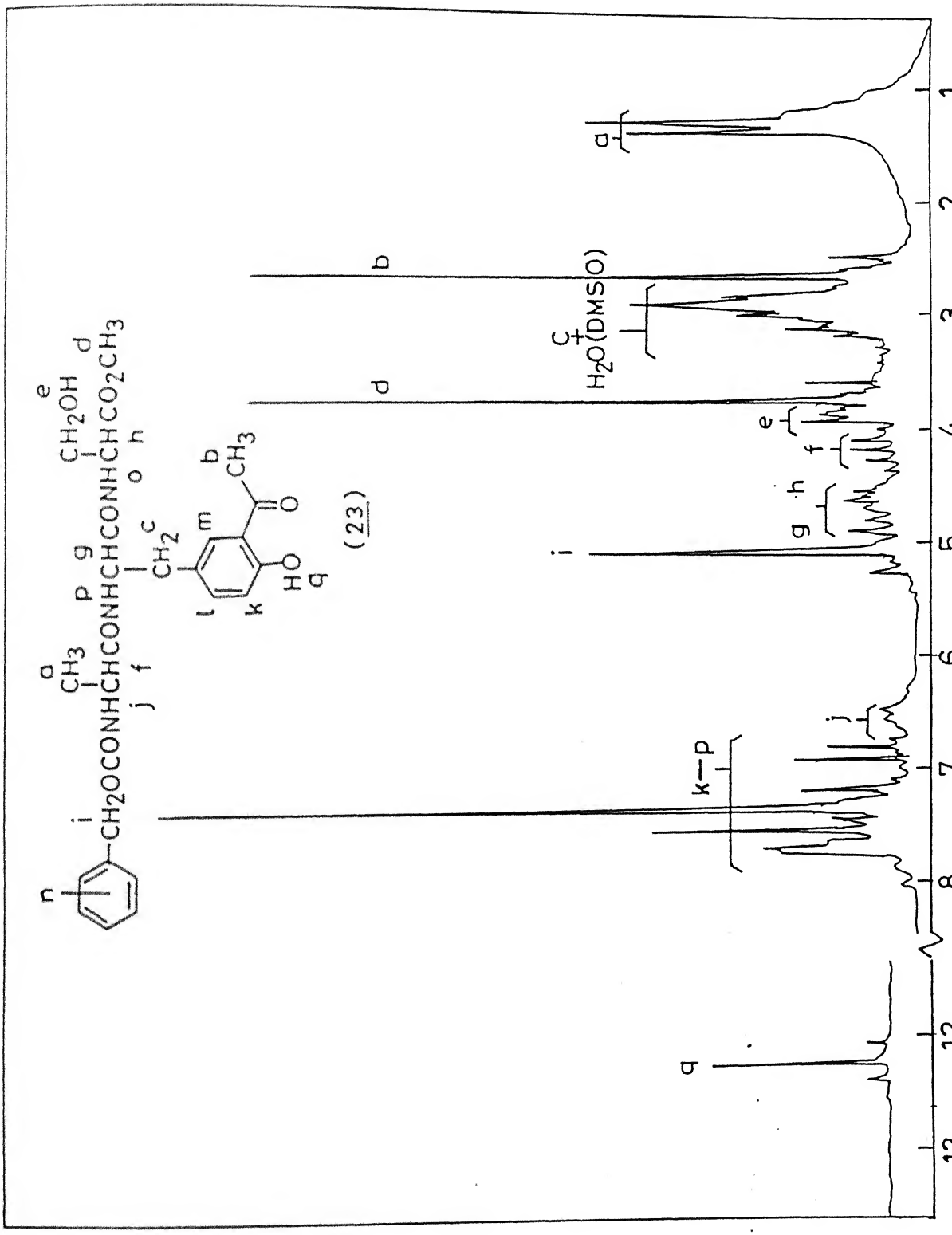

b
a
CH3
CH3
h
i
g
e
H3C
OCONHCHCONHCHCO2CH3
CH3
f
CH2d
k
l
c
j
COCH3
OH
m
(16)
b
e
c
a
m
l
k
j
i
g+h
f
d
13
12
8
7
6
5
4
3
2
1
PPM(δ), 80MHz (CDCl3)

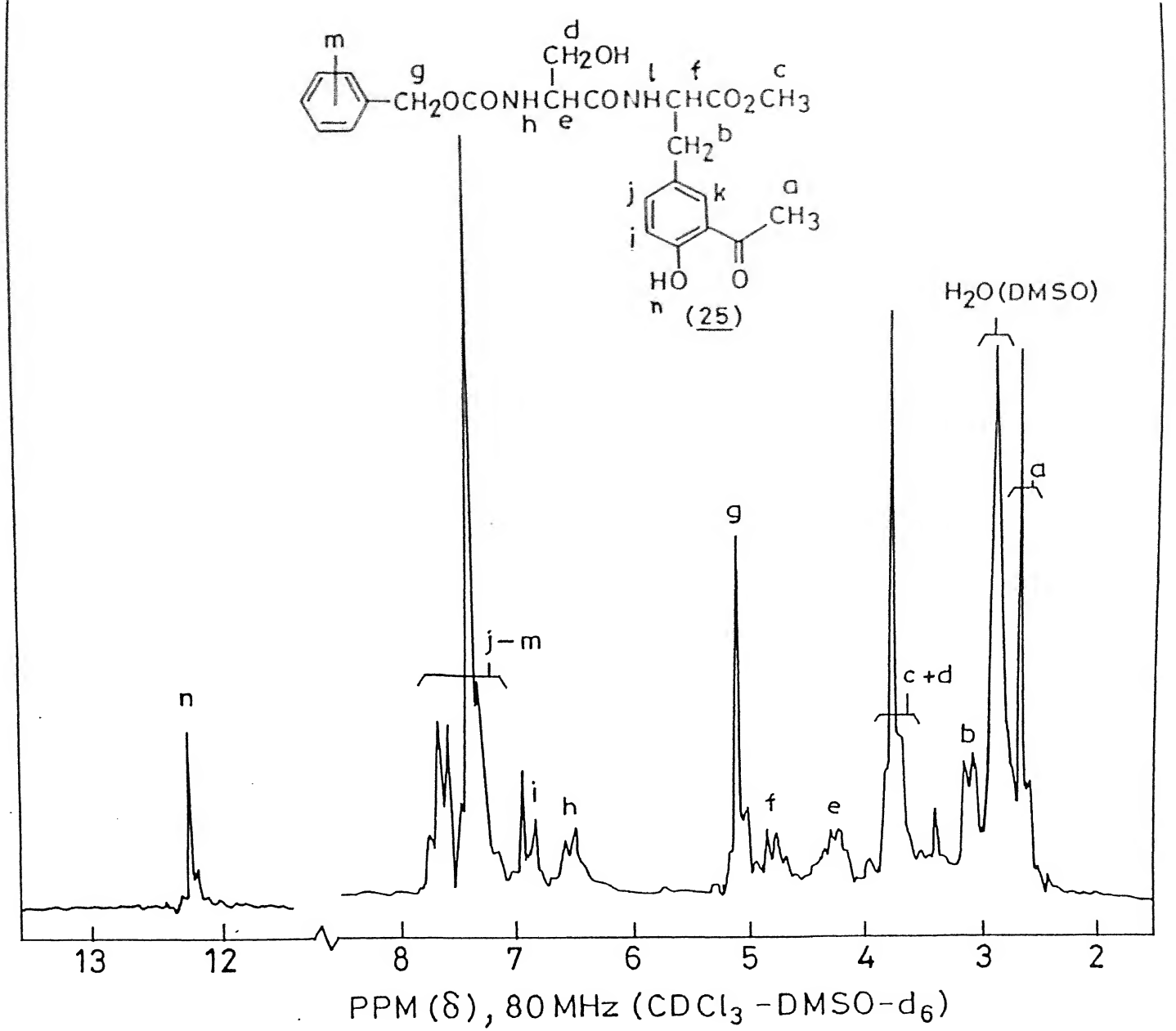

b
a
f
CH3
CH3
o h
CH2OH
H3C–C–OCONHCHCONHCHCONHCHCONHCHCO2CH3
CH3
j g
n i e
CH2 d
l
m
c
k
CH3
HO
O
P
(19)
b
e
c
a
P
k – o
f
d
j
h+i
g
imp
13
12
8
7
6
5
4
3
2
1
PPM(δ), 80MHz (CDCl3)

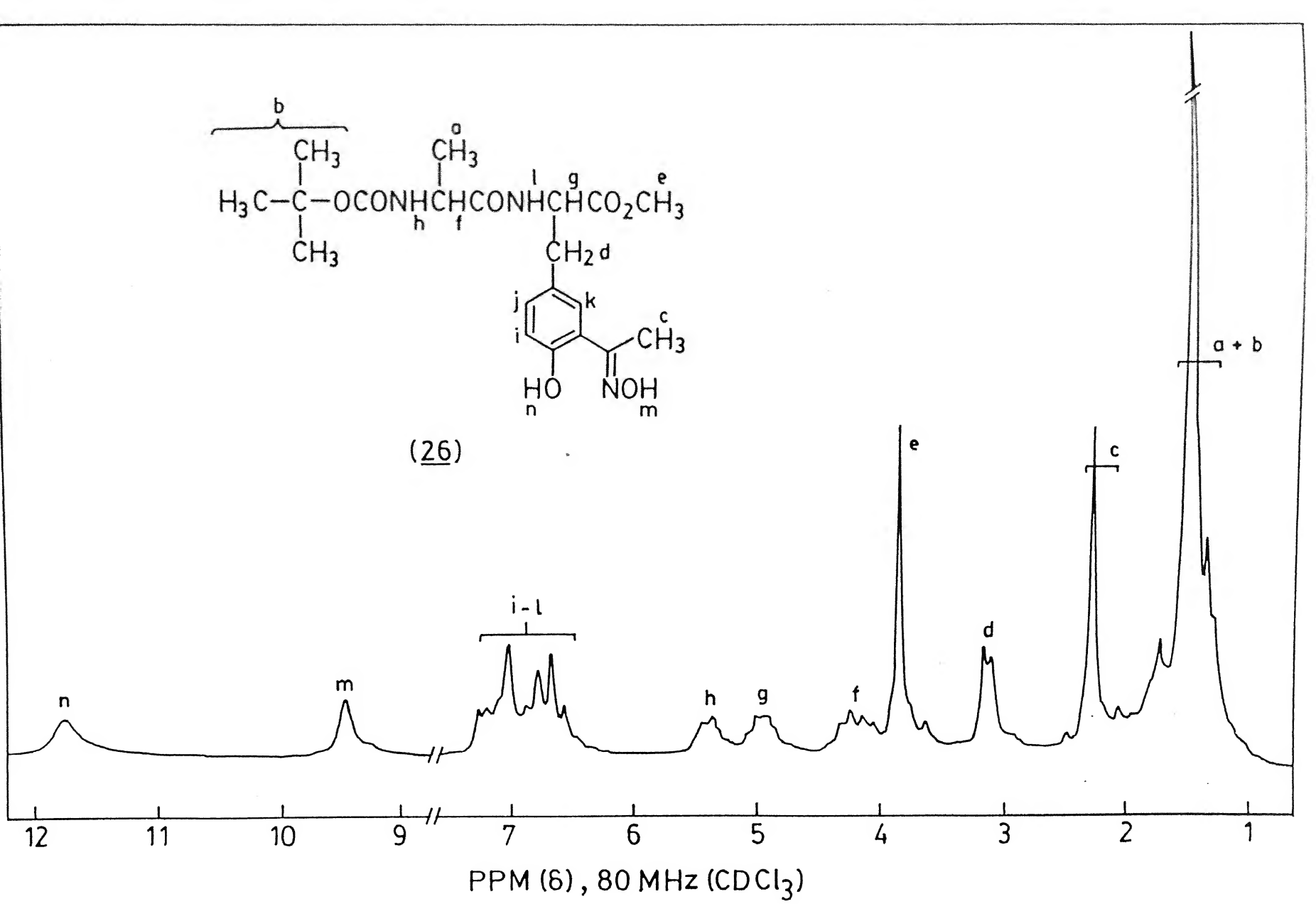

e
c d b f
CH2O CONH CH COOH
CH3
a
(20)
e
c
a
f
d
b
10 9 8 7 6 5 4 3 2 1
PPM (δ), 80 MHz (CDCl3)

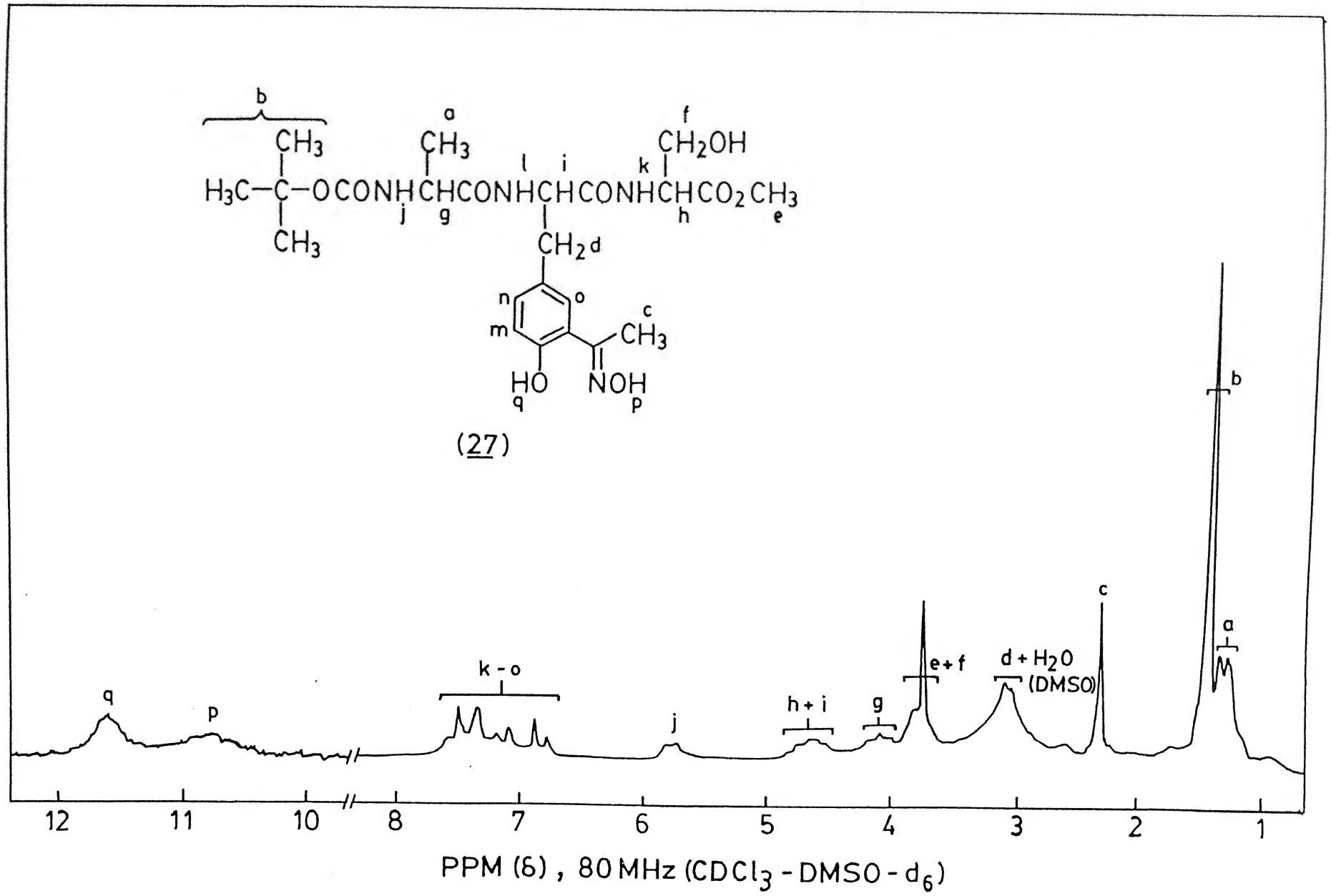

PPM (δ), 80 MHz ($CDCl_3$)
1
2
3
4
5
6
7
8
13
14
15
a
b
c
d
e
f
g
h
i
j
k
l
m
n
CH_3
$CH_2OCONHCHCONHCHCO_2CH_3$
CH_2
HO
O

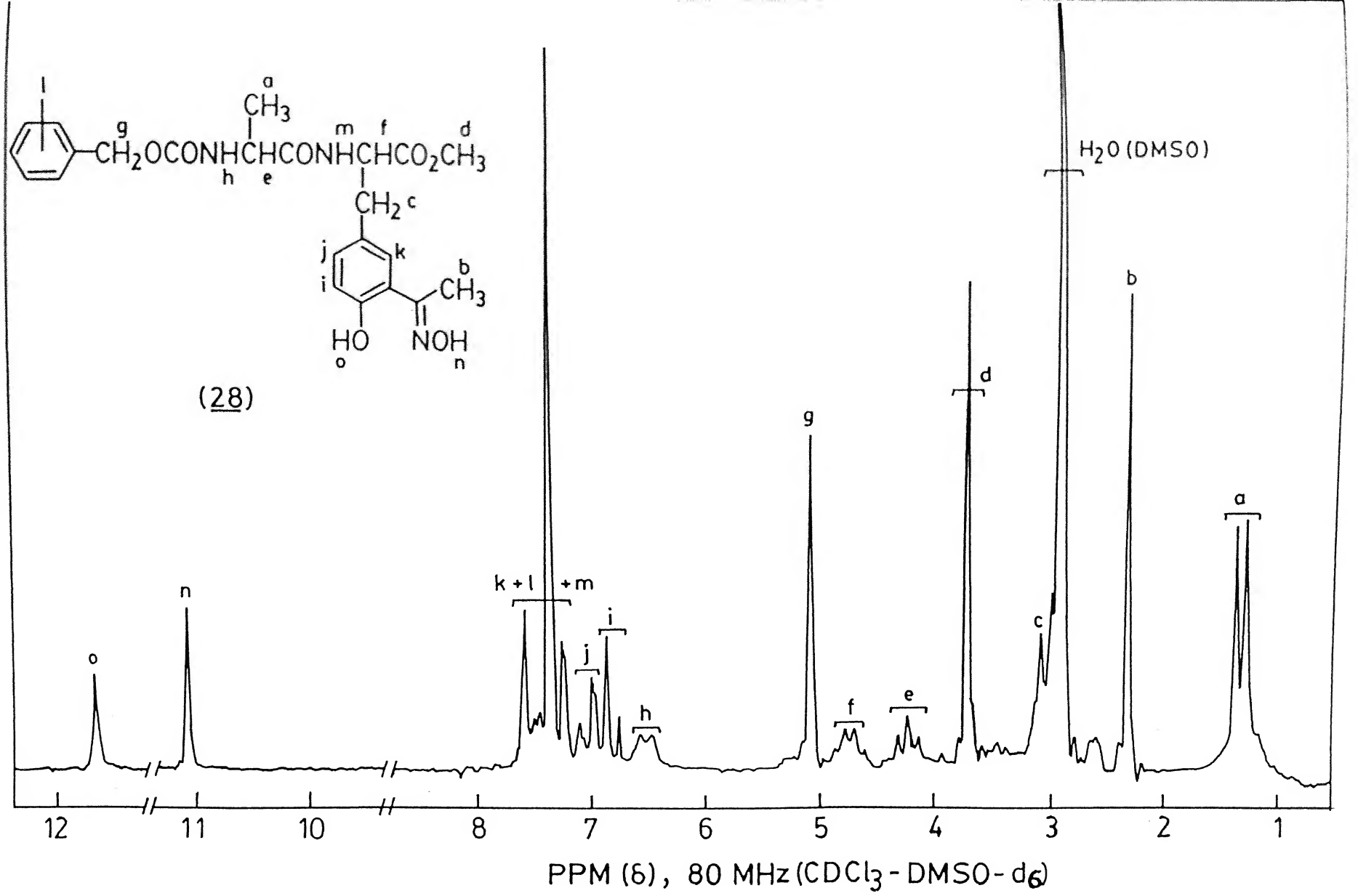
(21)

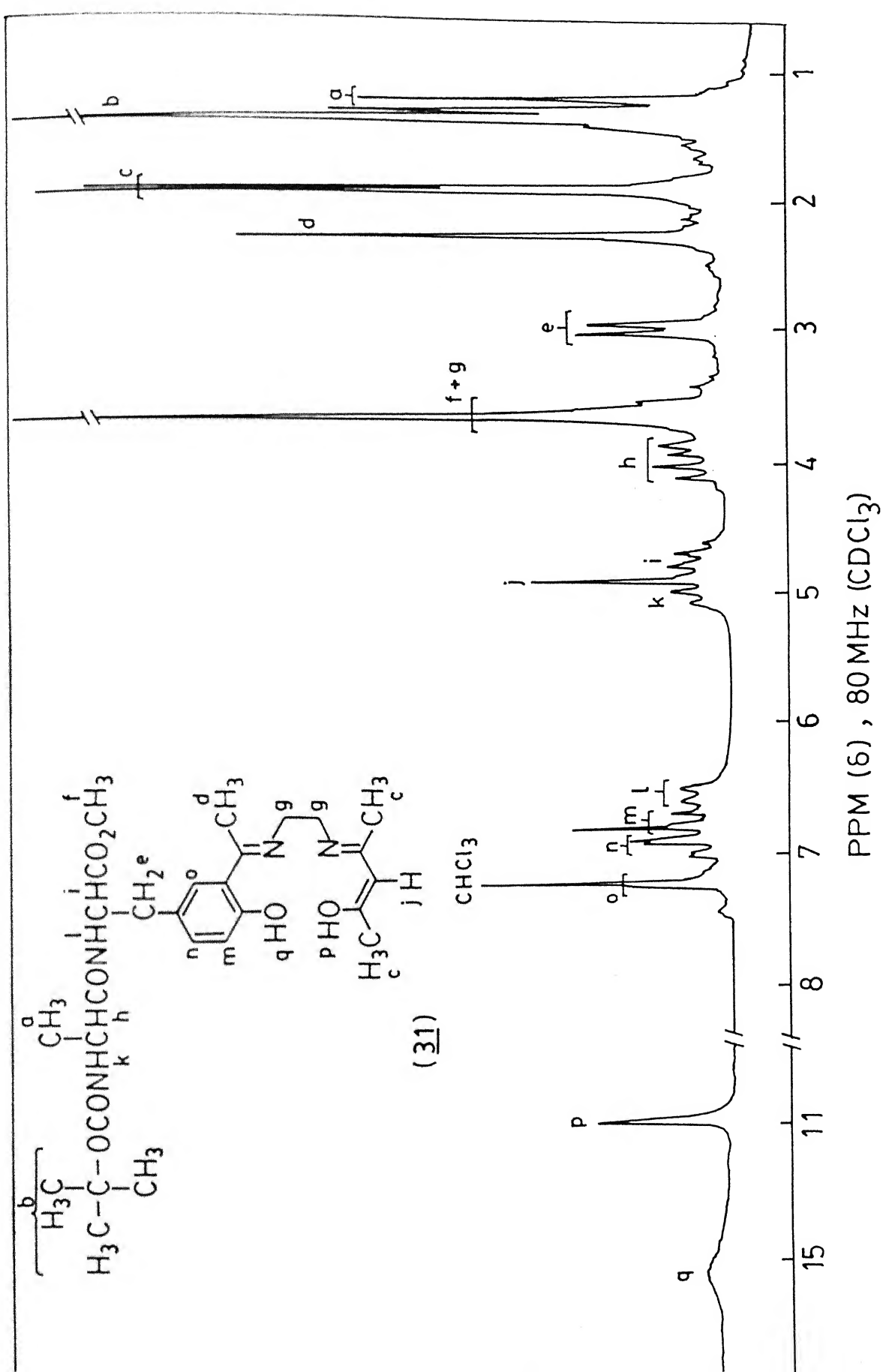

PPM (δ), 80 MHz (CDCl3 - DMSO-d6)
(23)
H2O(DMSO)

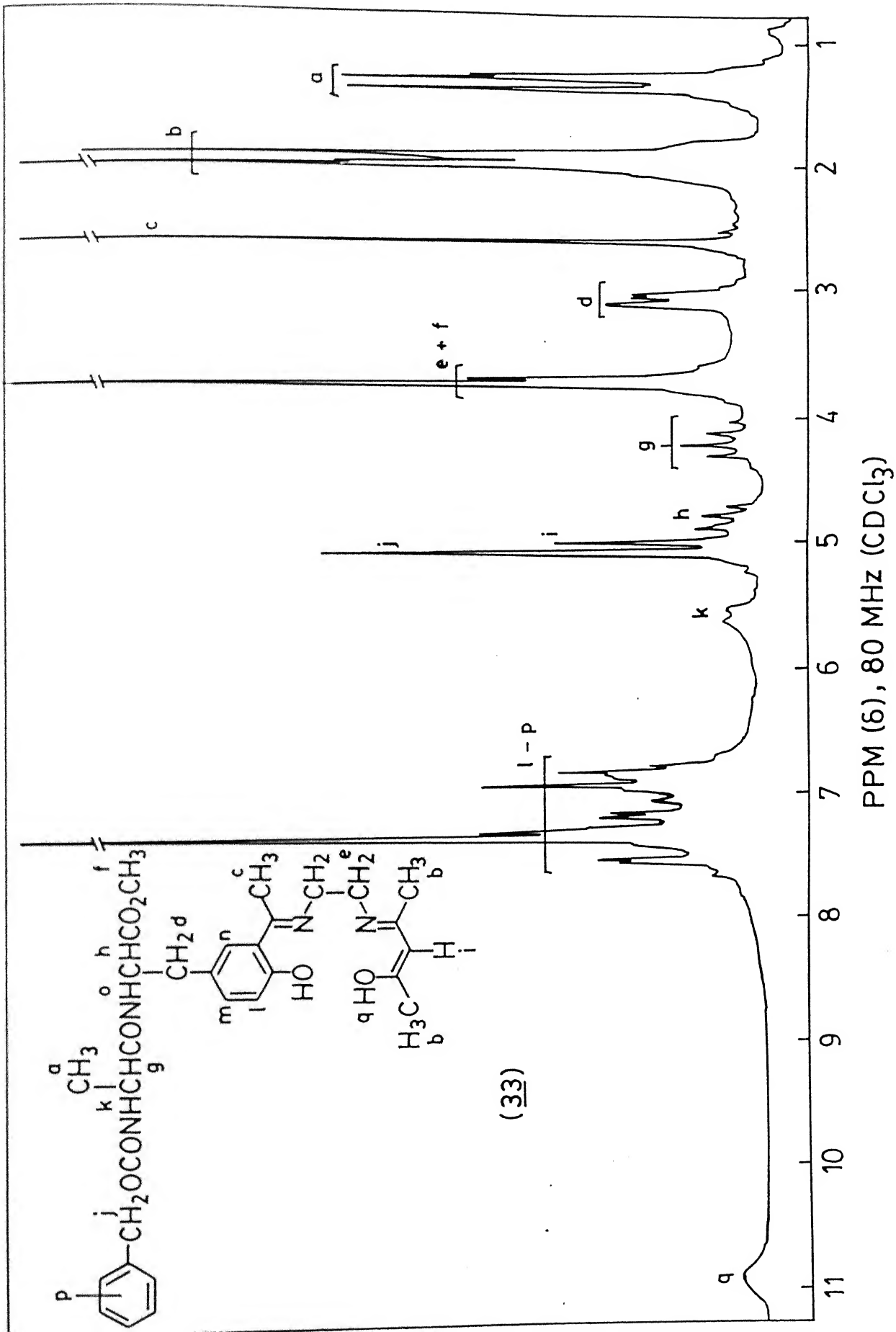

m
d
CH2OH
g
l
f
c
CH2OCONHCHCONHCHCO2CH3
h
e
CH2
b
j
k
a
CH3
i
HO
O
n
(25)
H2O(DMSO)
a
g
j–m
c+d
n
b
i
h
f
e
13
12
8
7
6
5
4
3
2
PPM (δ), 80 MHz (CDCl3 –DMSO-d6)

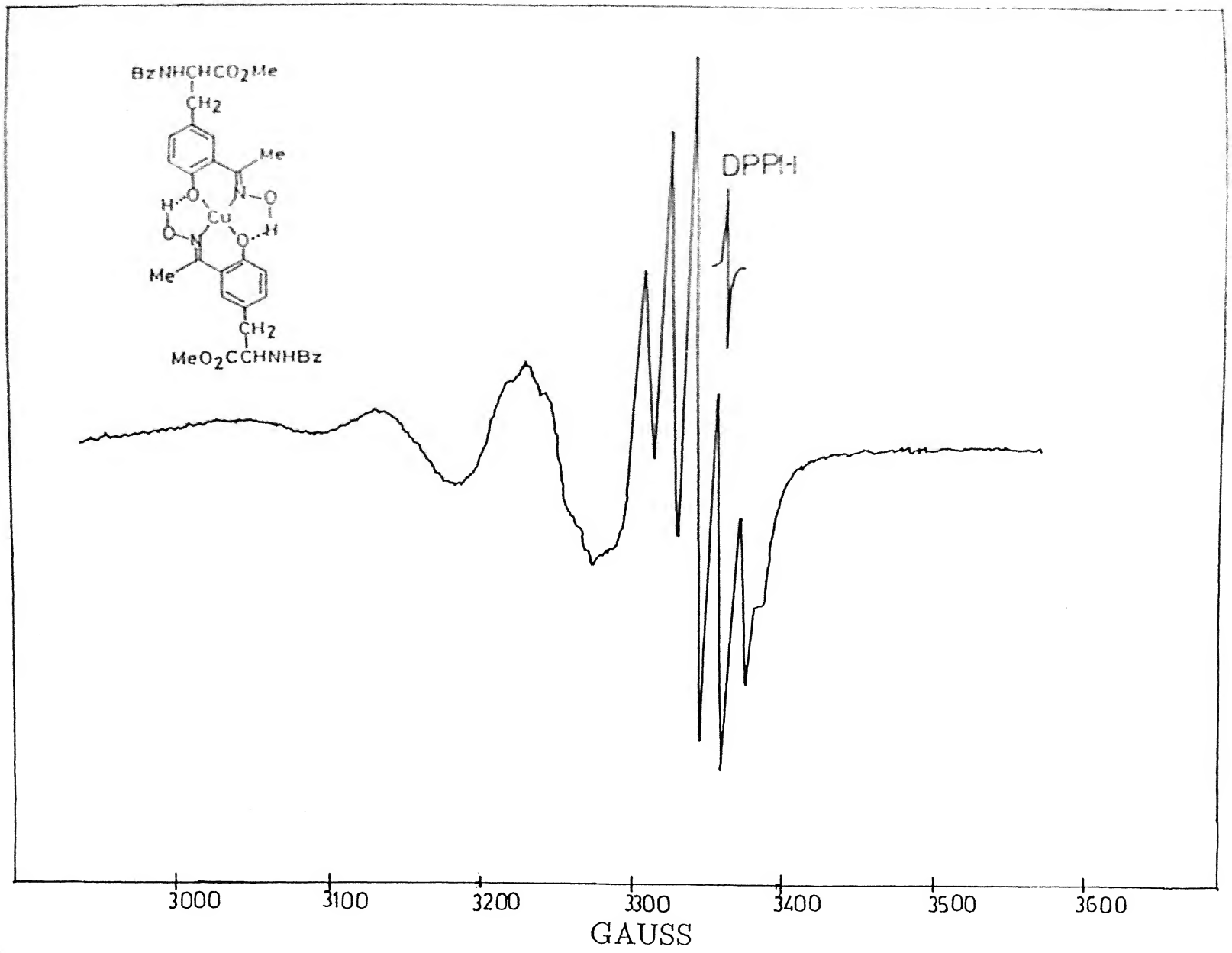

b
a
CH3
CH3
H3C–C–OCONHCHCONHCHCO2CH3
CH3
h
f
l
g
e
CH2 d
j
k
i
c
CH3
HO
n
NOH
m
(26)
a + b
e
c
i - l
d
n
m
h
g
f
12
11
10
9
7
6
5
4
3
2
1
PPM (δ), 80 MHz (CDCl3)


b
a
f
CH3
CH3
CH2OH
H3C—C—OCONHCHCONHCHCONHCHCO2CH3
CH3
j g l i k h e
CH2 d
n
o
m
c
CH3
HO
NOH
q p
(27)
b
a
c
d + H2O (DMSO)
e + f
h + i
g
k - o
q
p
j
12
11
10
8
7
6
5
4
3
2
1
PPM (δ), 80 MHz (CDCl3 - DMSO - d6)

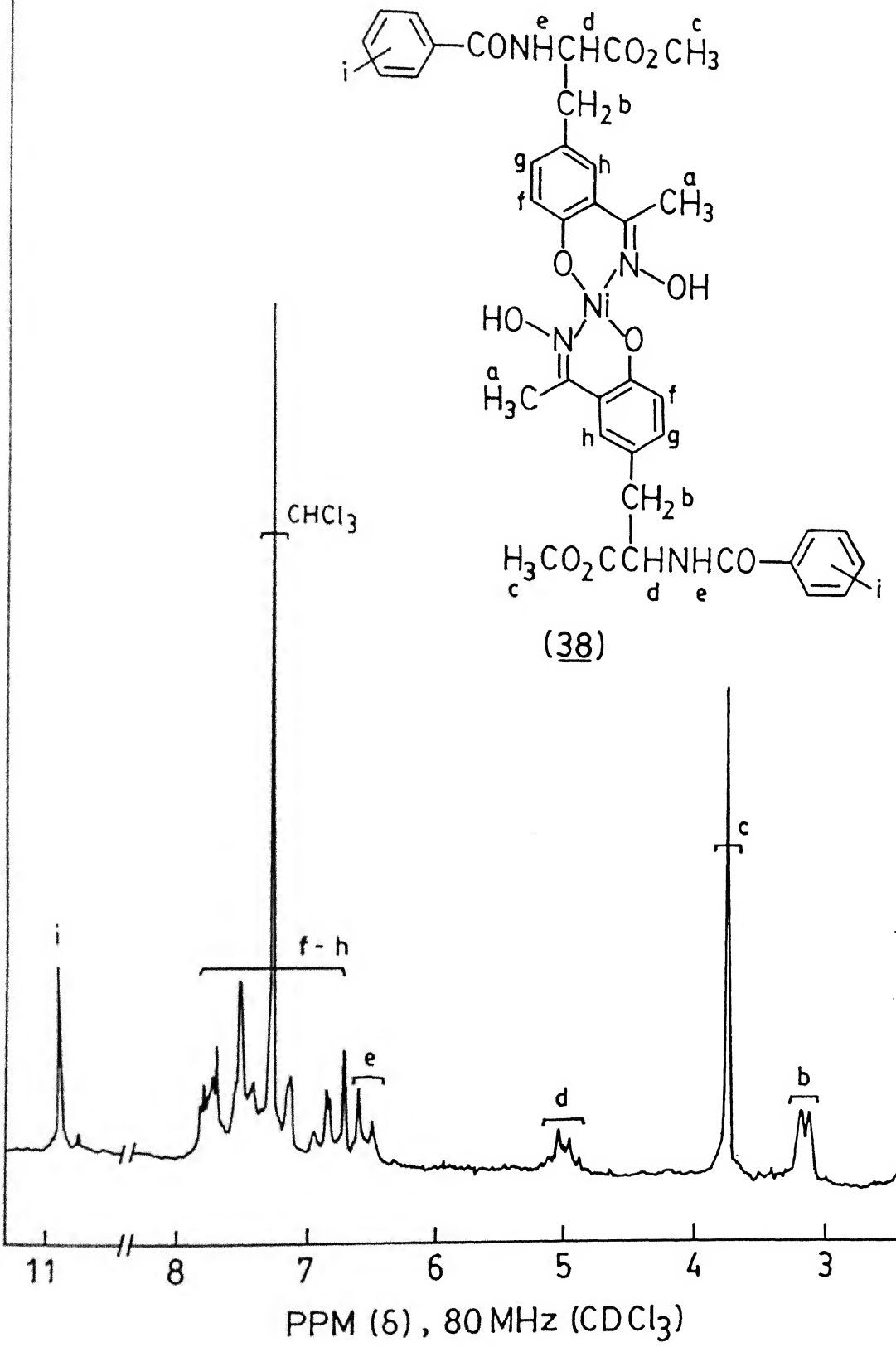

l
a
CH3
g
m
f
d
CH2OCONHCHCONHCHCO2CH3
h
e
CH2 c
j
k
i
b
CH3
HO
o
NOH
n
(28)
H2O (DMSO)
b
d
g
a
k + l + m
n
i
c
j
o
h
f
e
12
11
10
8
7
6
5
4
3
2
1
PPM (δ), 80 MHz (CDCl3 - DMSO - d6)

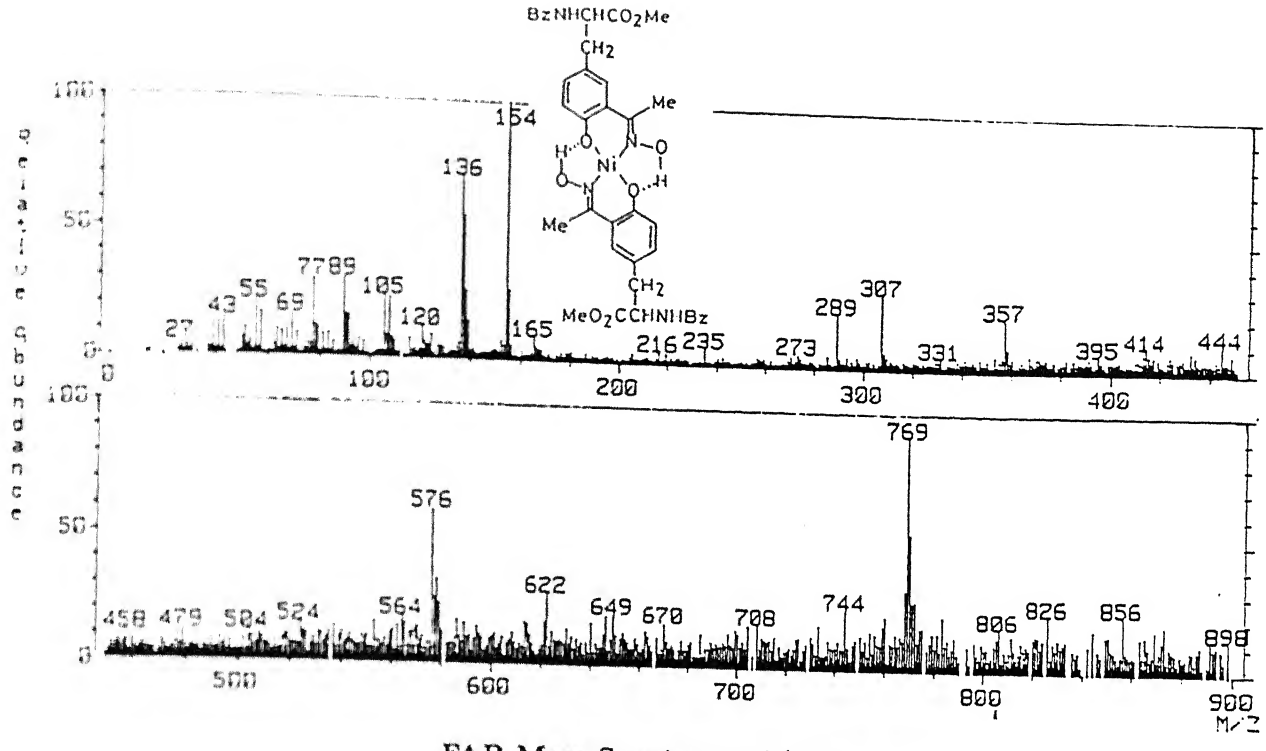

PPM (δ), 80 MHz ($CDCl_3$)
(31)
$CHCl_3$
f + g


PPM (δ), 80 MHz ($CDCl_3$)
(33)

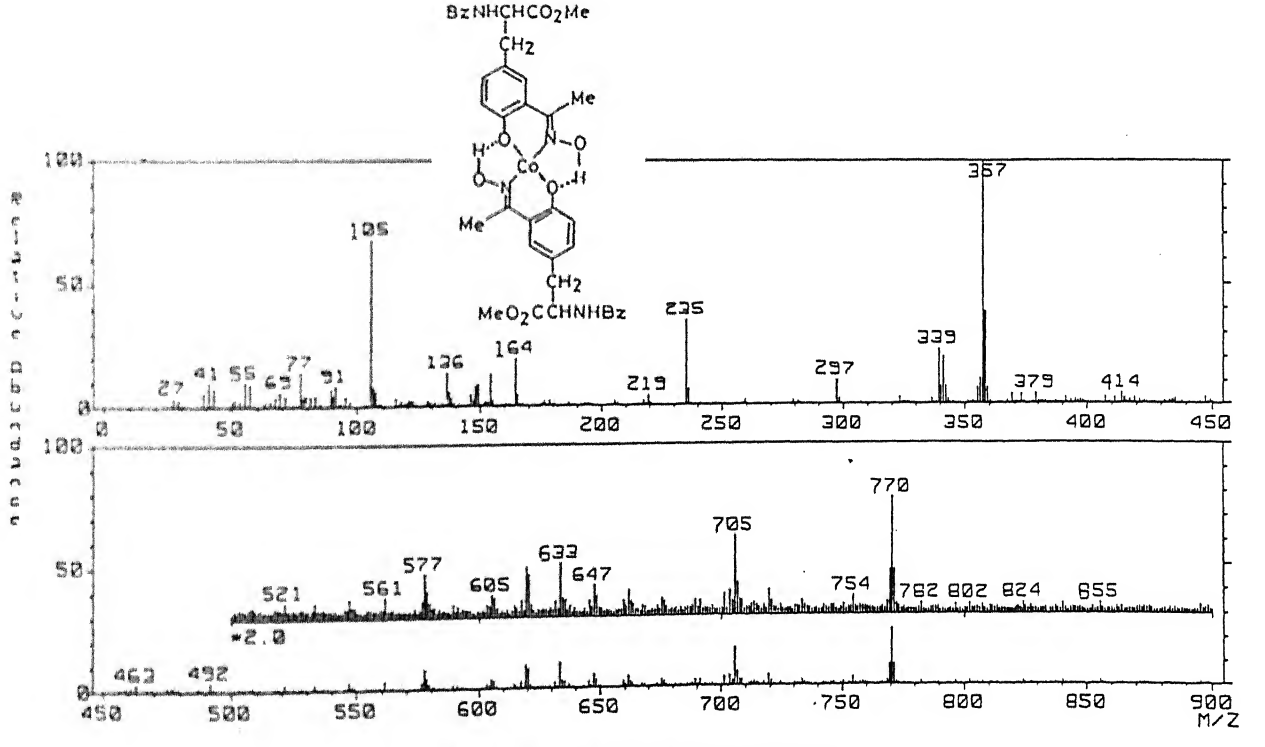


EPR Spectrum of (37) at Room temperature

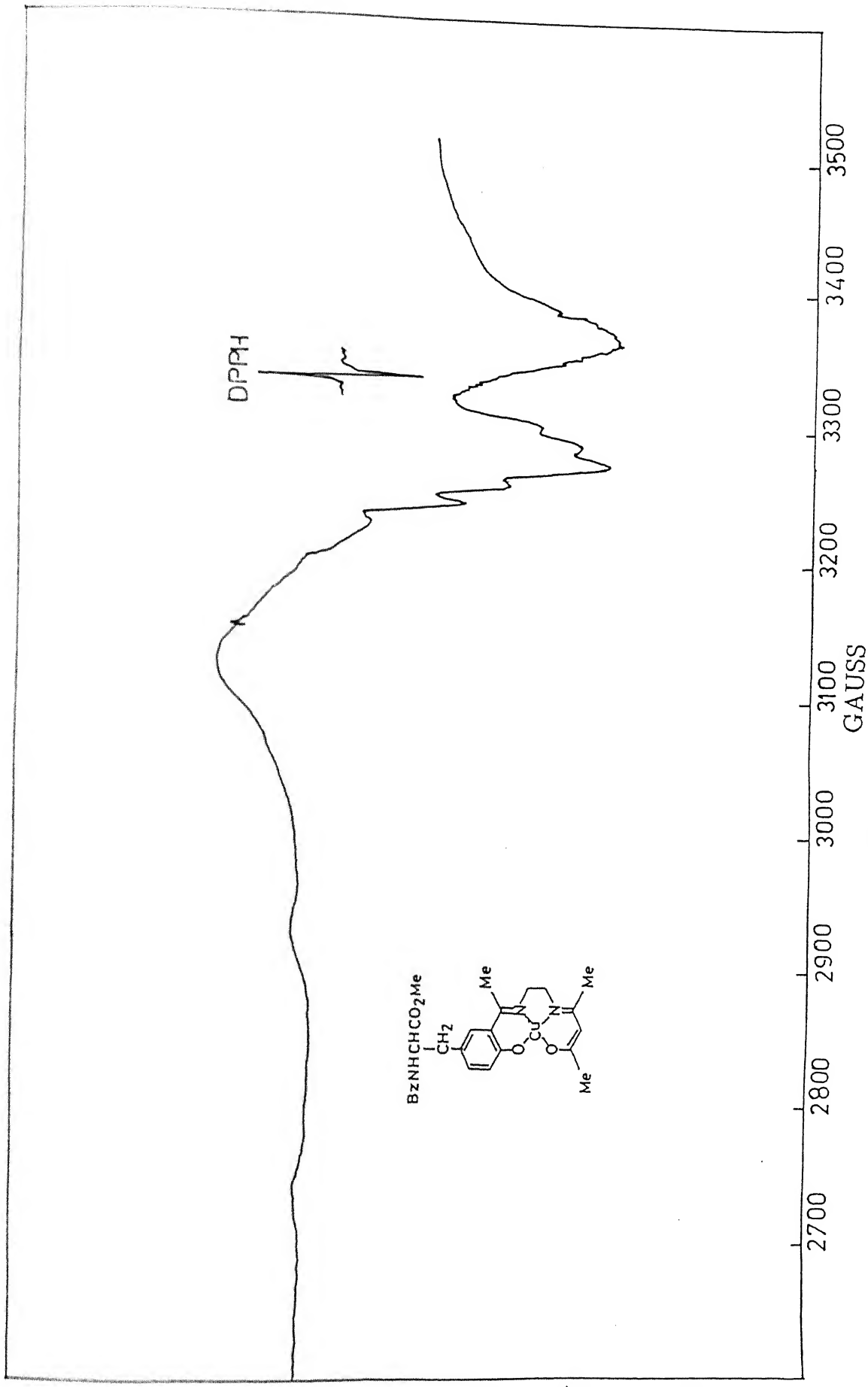

CONHCHCO2CH3
CH2 b
CH3
OH
Ni
HO
H3C
CHCl3
H3CO2CCHNHCO
(38)
i
f - h
e
d
c
b
a
11
8
7
6
5
4
3
2
PPM (δ), 80 MHz (CDCl3)



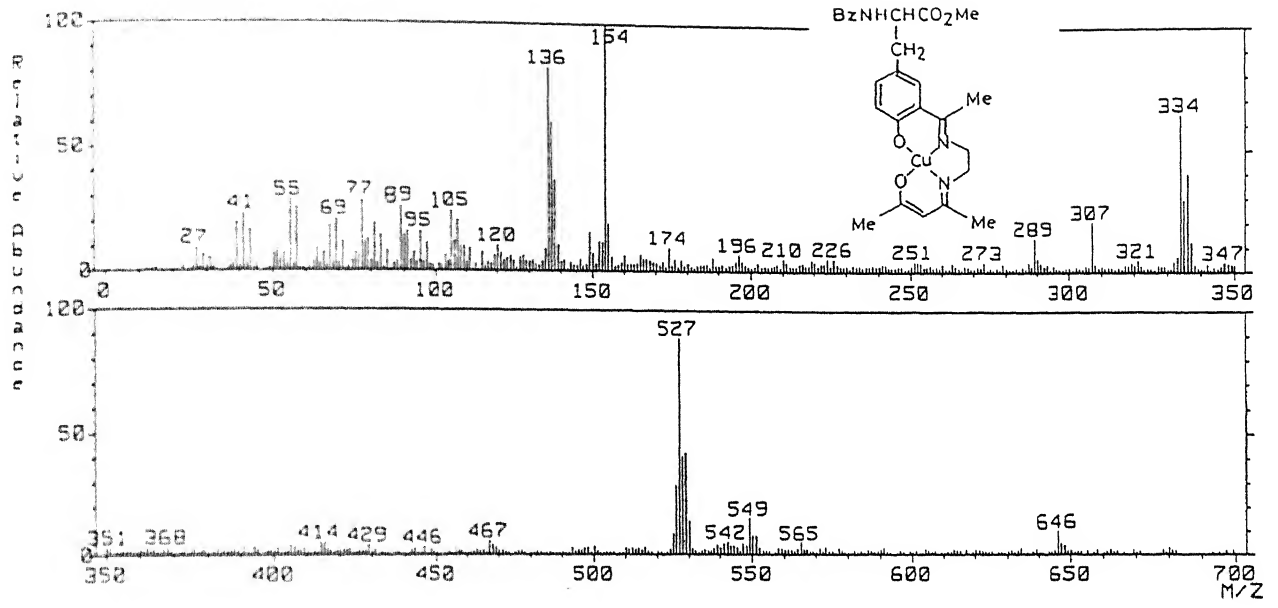
FAB Mass Spectrum of (38)



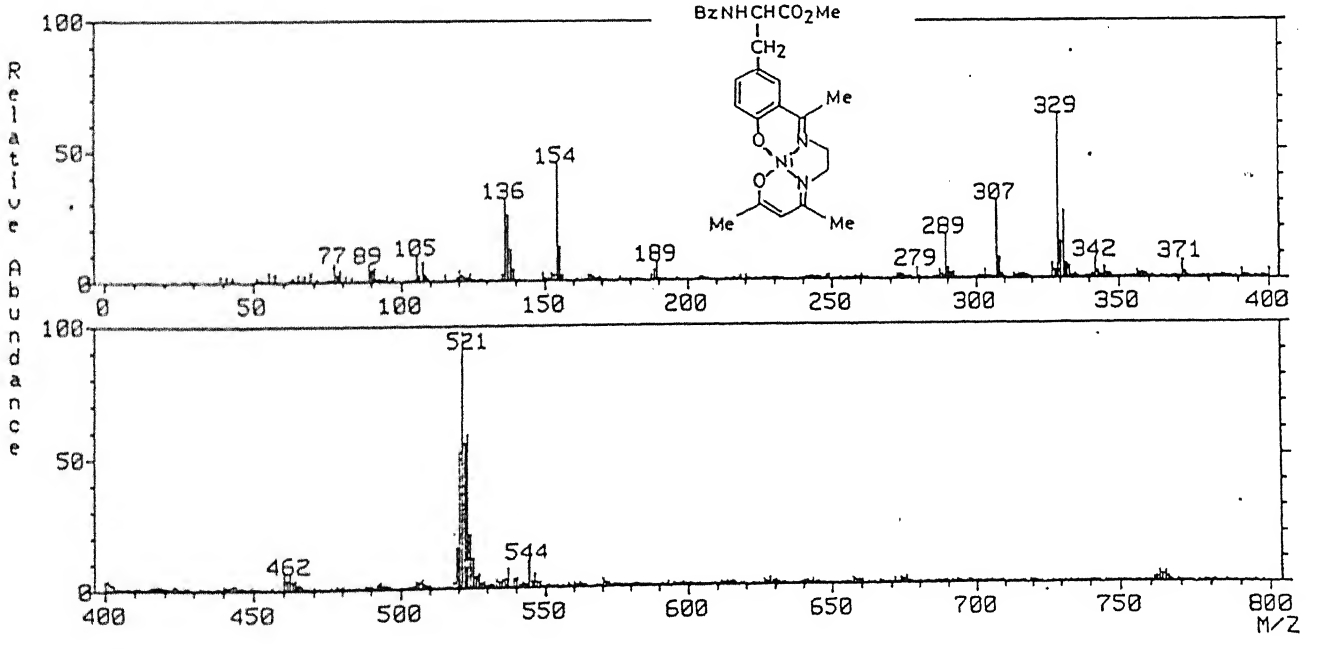
FAB Mass Spectrum of (39)



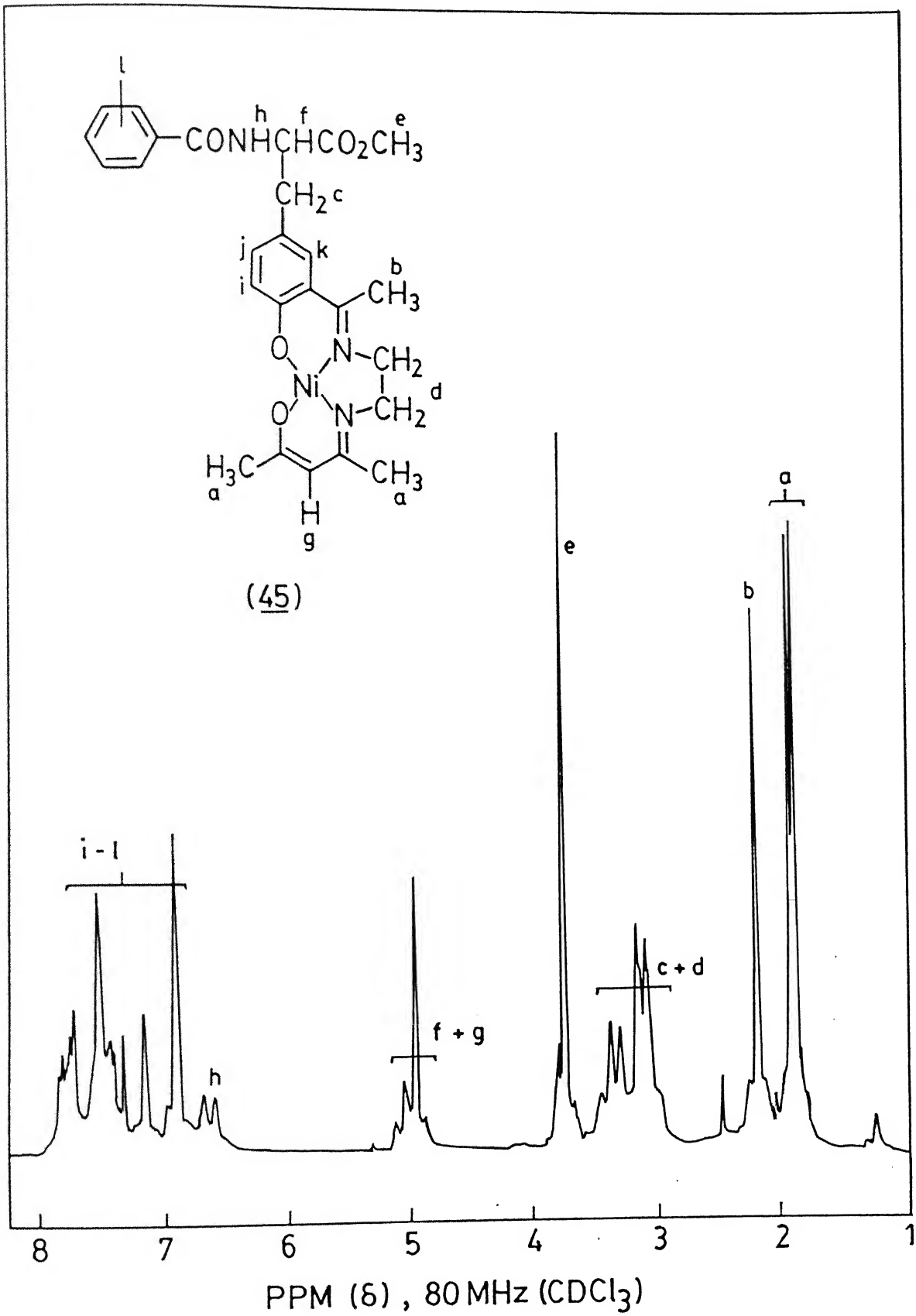
EPR Spectrum of (44) at -196°C

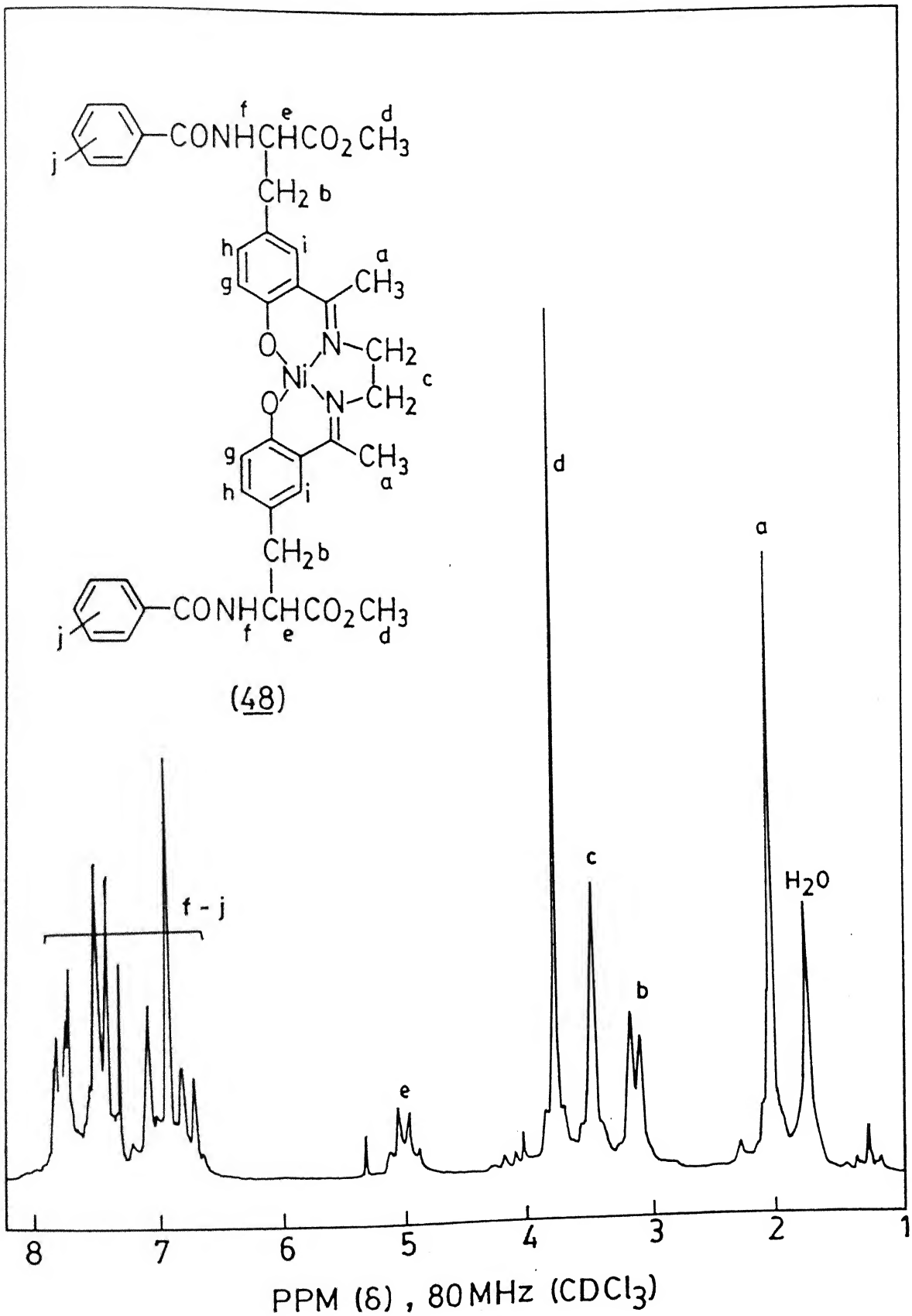


FAB Mass Spectrum of (44)

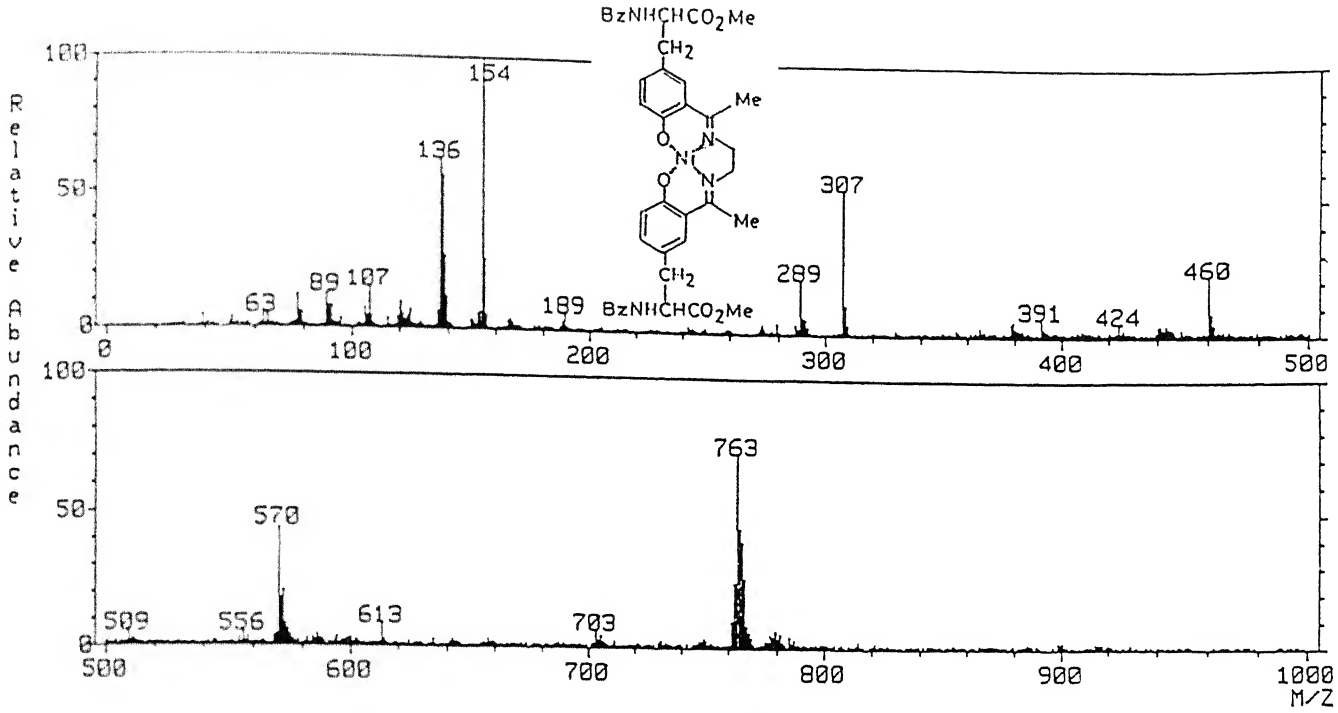


FAB Mass Spectrum of (45)

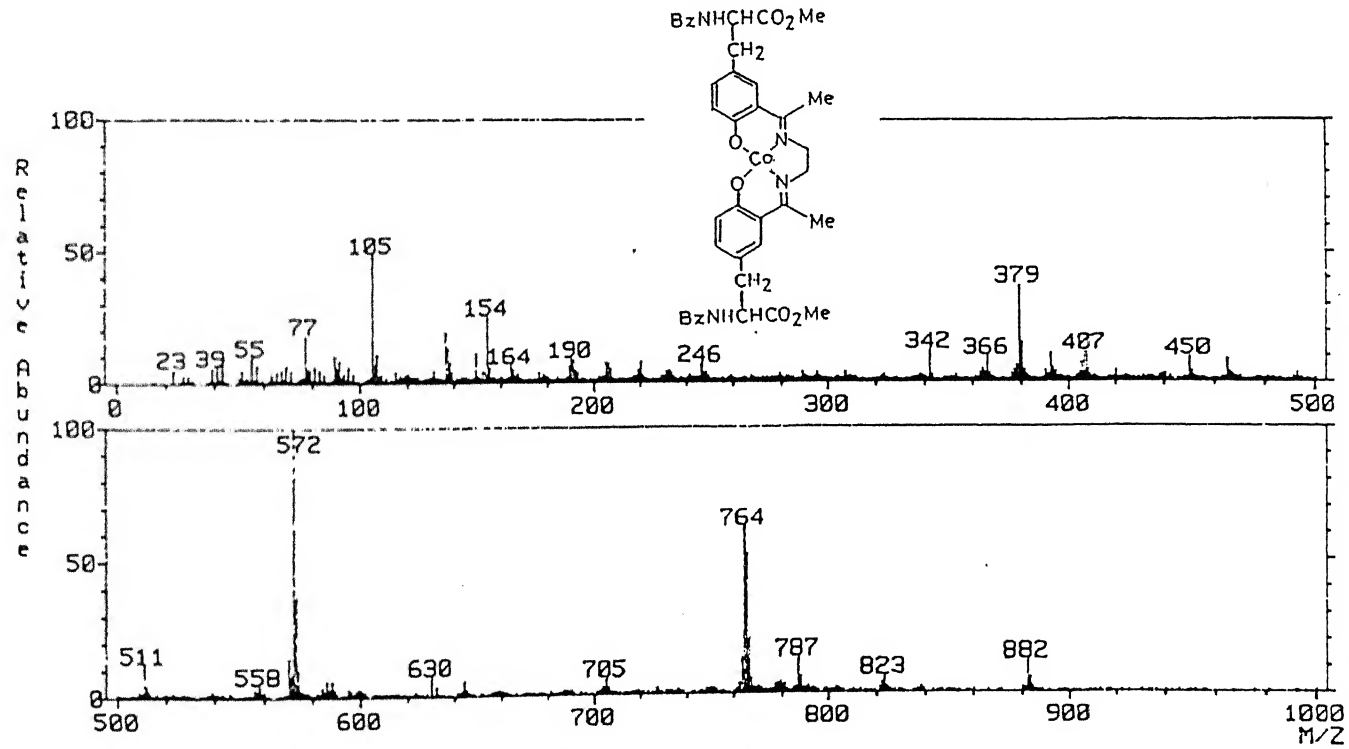

l
h
f
e
CONHCHCO2CH3
CH2 c
j
k
i
b
CH3
O
N
CH2
Ni
d
O
N
CH2
H3C
a
CH3
a
H
g
(45)
a
b
e
i - l
h
f + g
c + d
8
7
6
5
4
3
2
1
PPM (δ) , 80 MHz (CDCl3)


CONHCHCO2CH3
CH2 b
CH3
CH2
CH2
Ni
O
N
CH3
CH2b
CONHCHCO2CH3
f - j
d
a
c
H2O
b
e
8
7
6
5
4
3
2
1
PPM (δ) , 80 MHz (CDCl3)

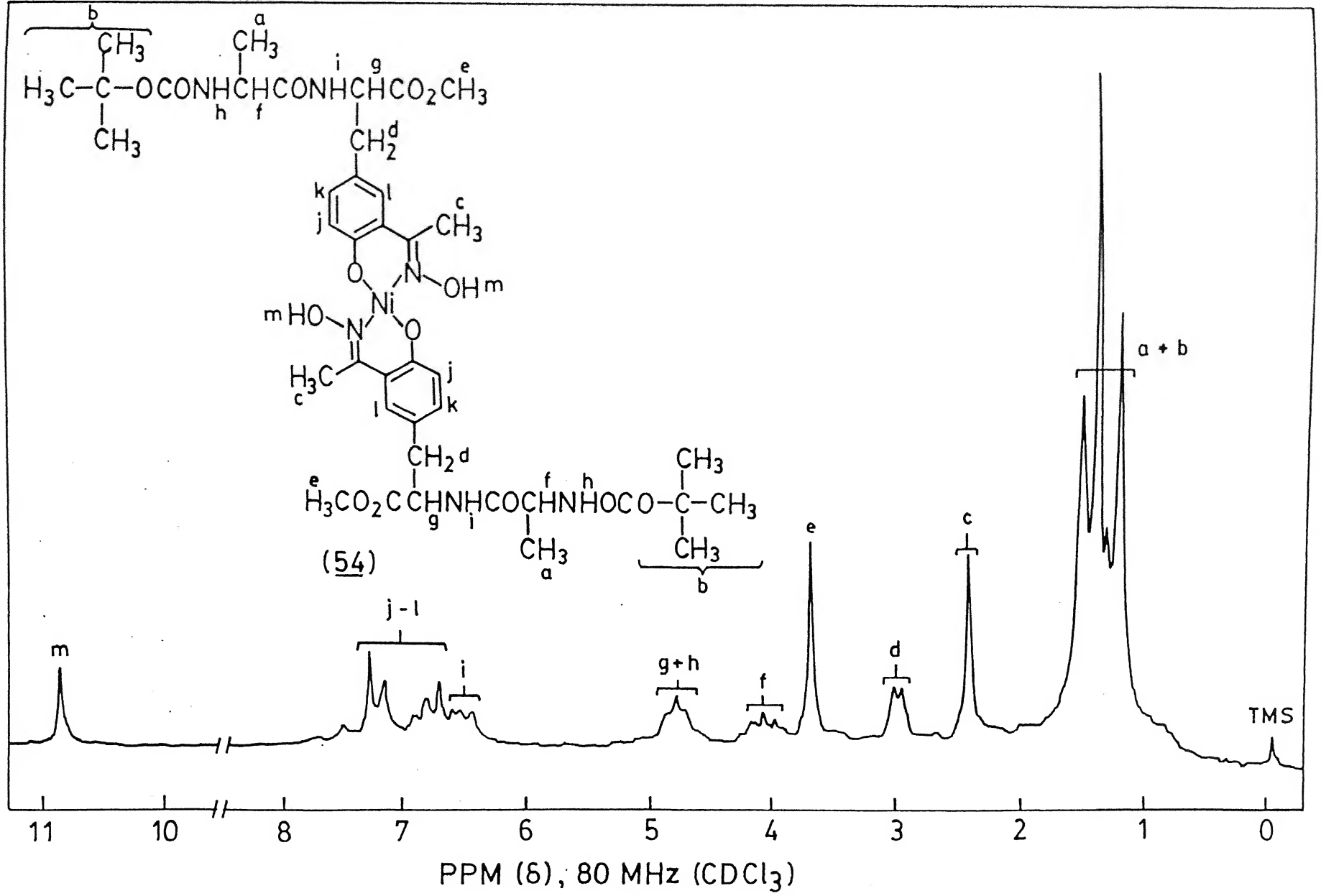
(48)

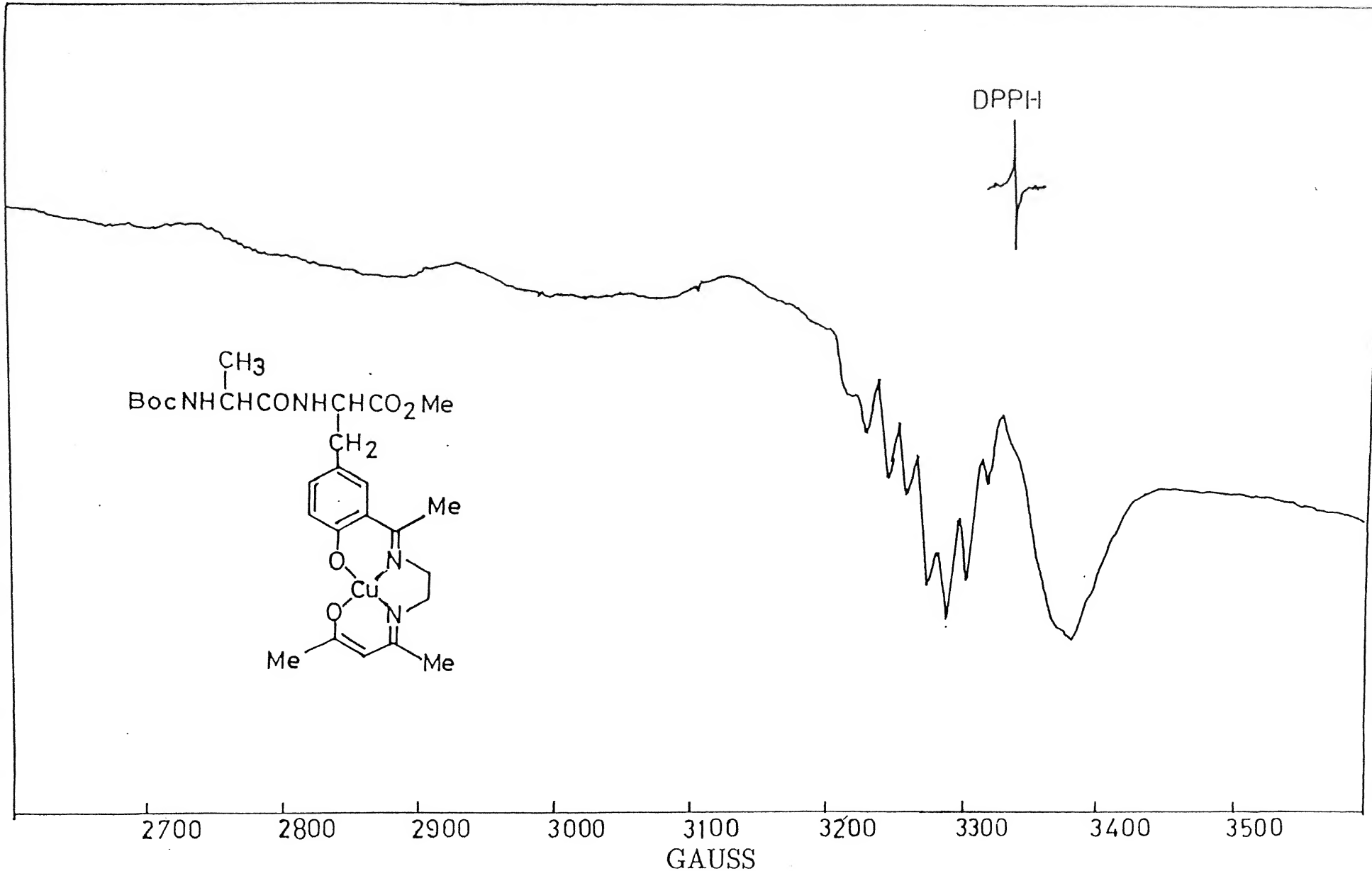


FAB Mass Spectrum of (48)

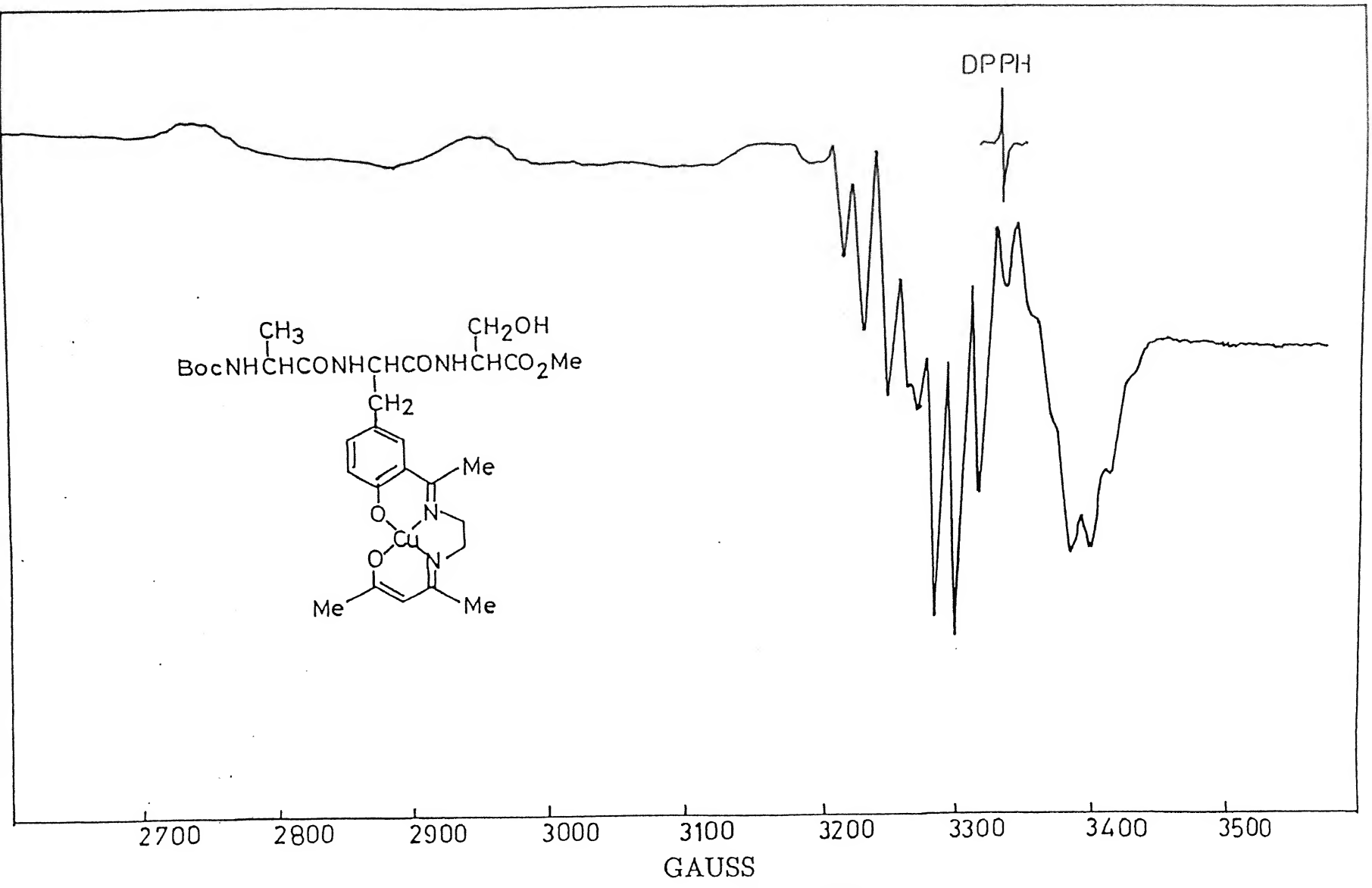


FAB Mass Spectrum of (49)

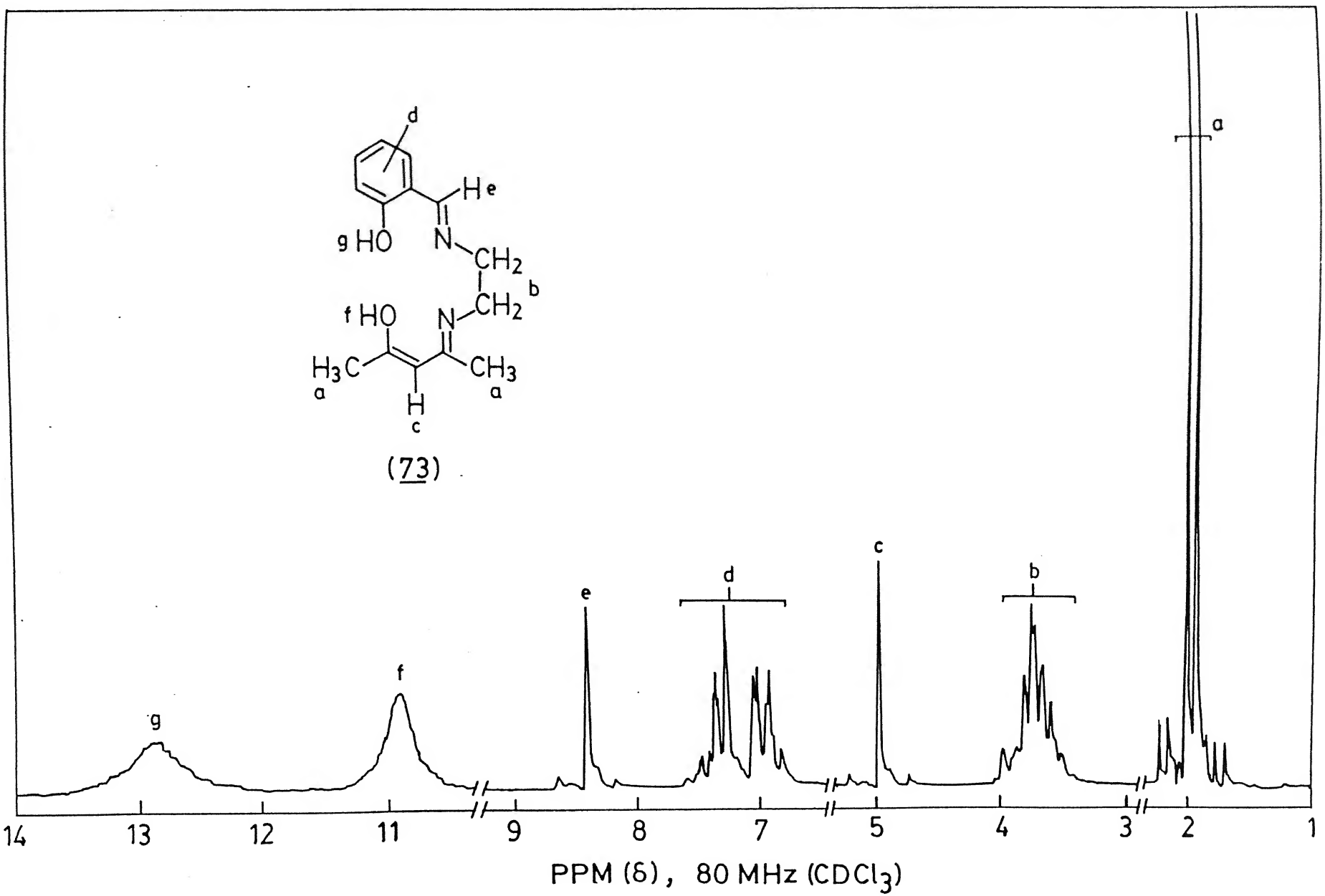

(54)
a + b
j - l
m
i
g + h
f
e
d
c
TMS
11
10
8
7
6
5
4
3
2
1
0
PPM (δ), 80 MHz (CDCl3)

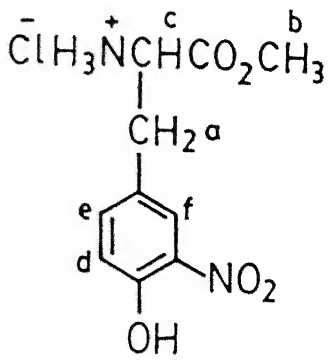


EPR Spectrum of (64) at -196°C



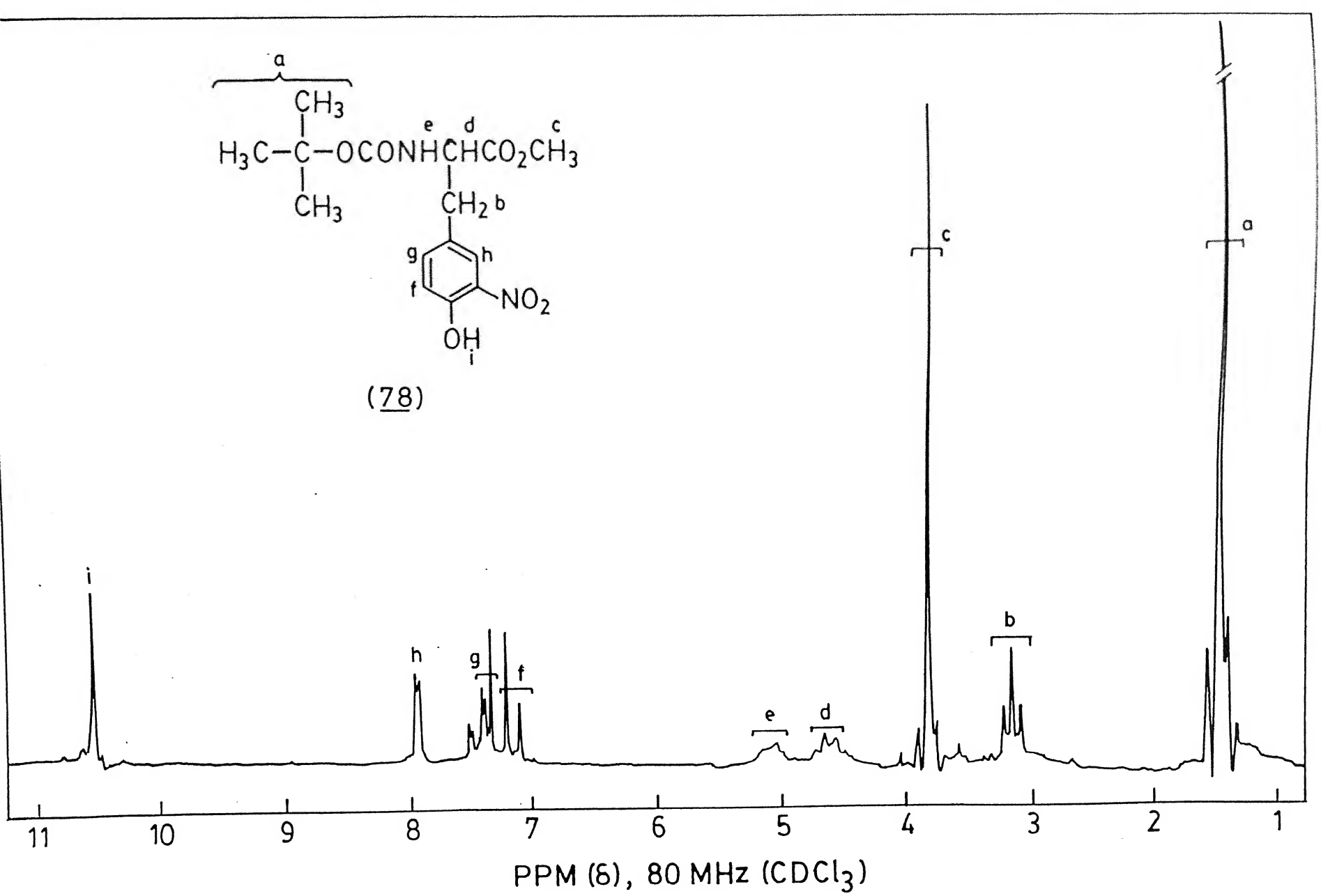
EPR Spectrum of (65) at -196°C

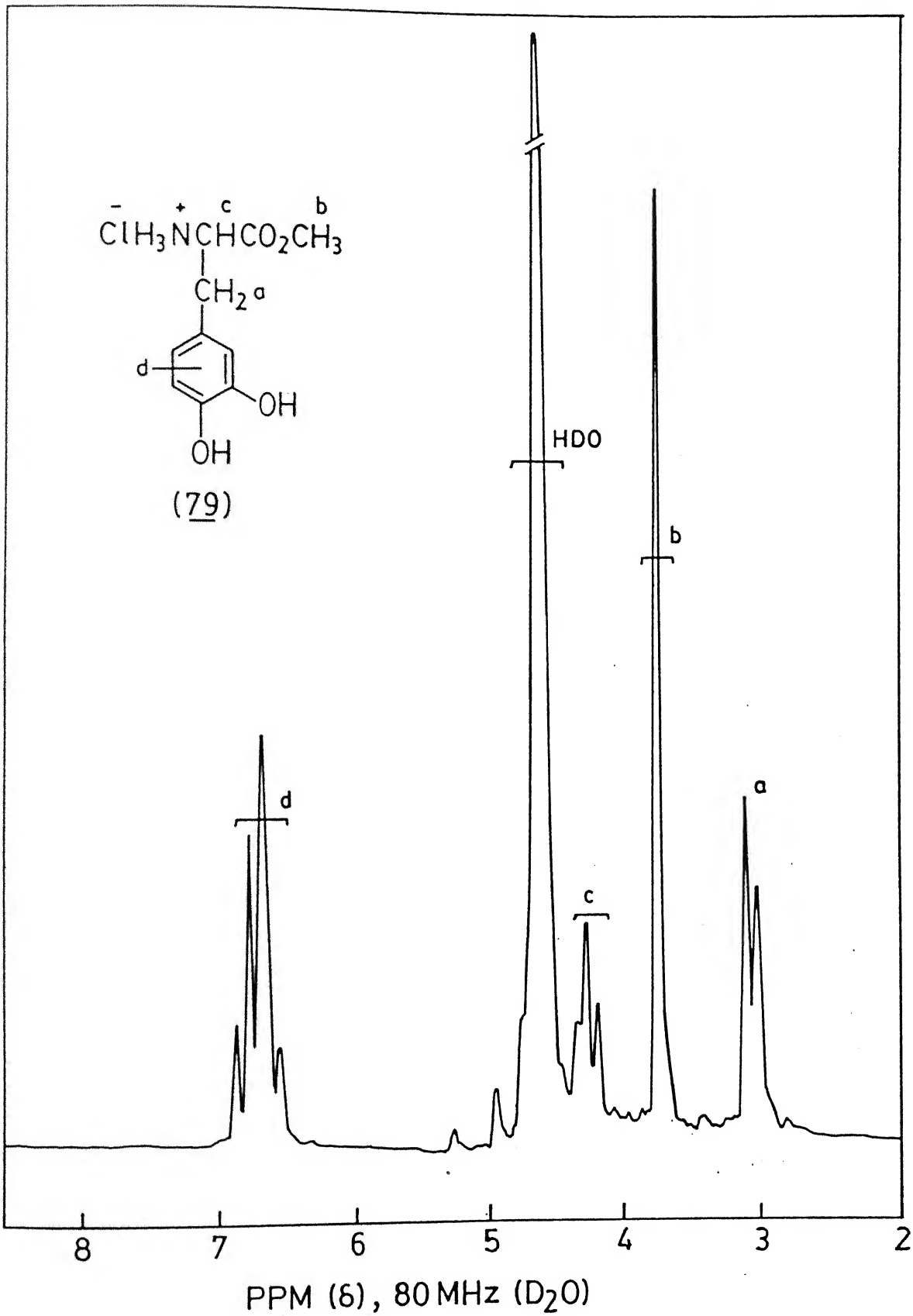

d
H e
g HO
N
CH2
b
CH2
f HO
N
H3C
a
CH3
a
H
c
(73)
a
c
d
b
e
f
g
14
13
12
11
9
8
7
5
4
3
2
1
PPM (δ), 80 MHz (CDCl3)


Cl⁻ H3N+ C CHCO2CH3
c
b
CH2 a
e
f
d
NO2
OH

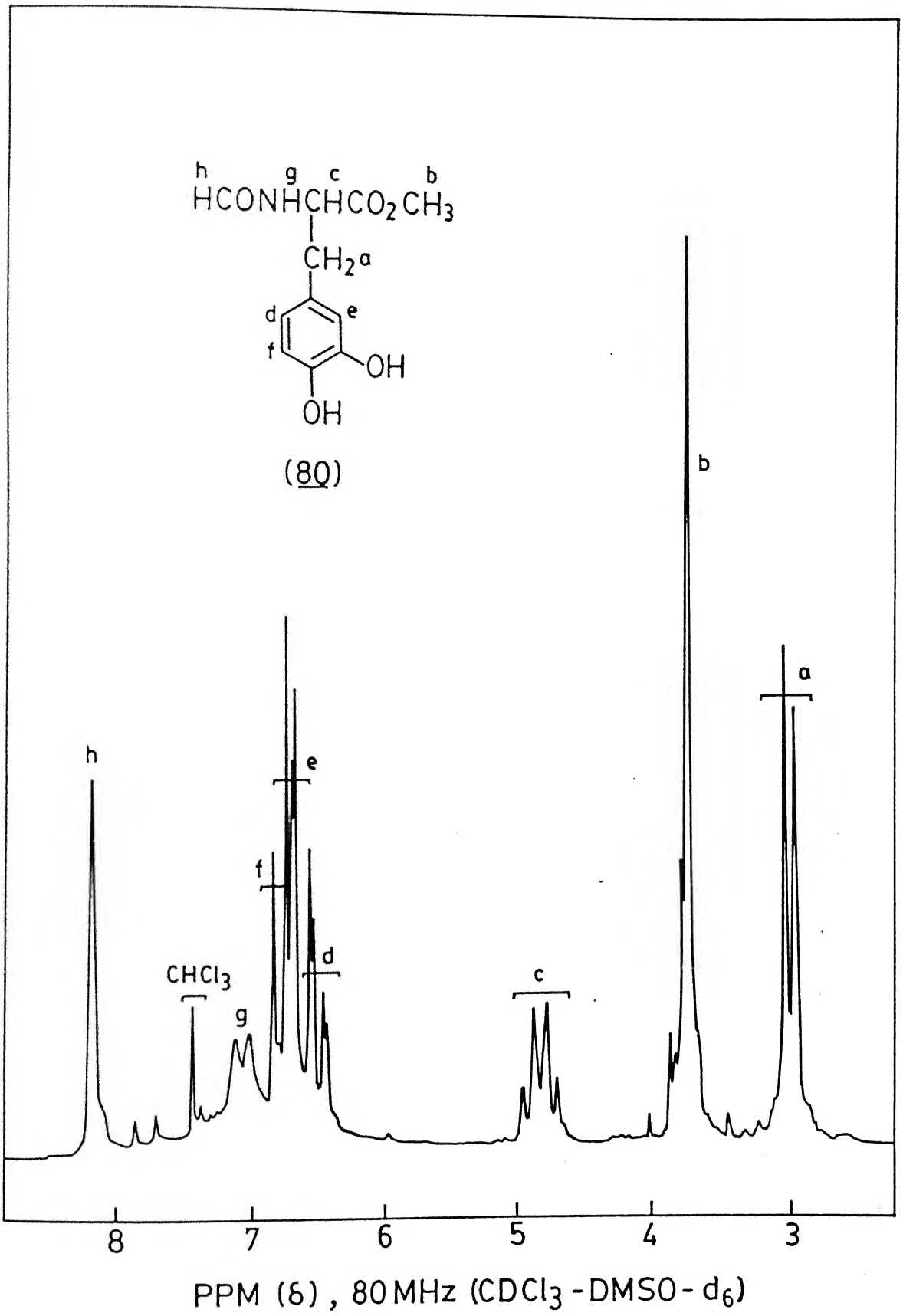
(77)

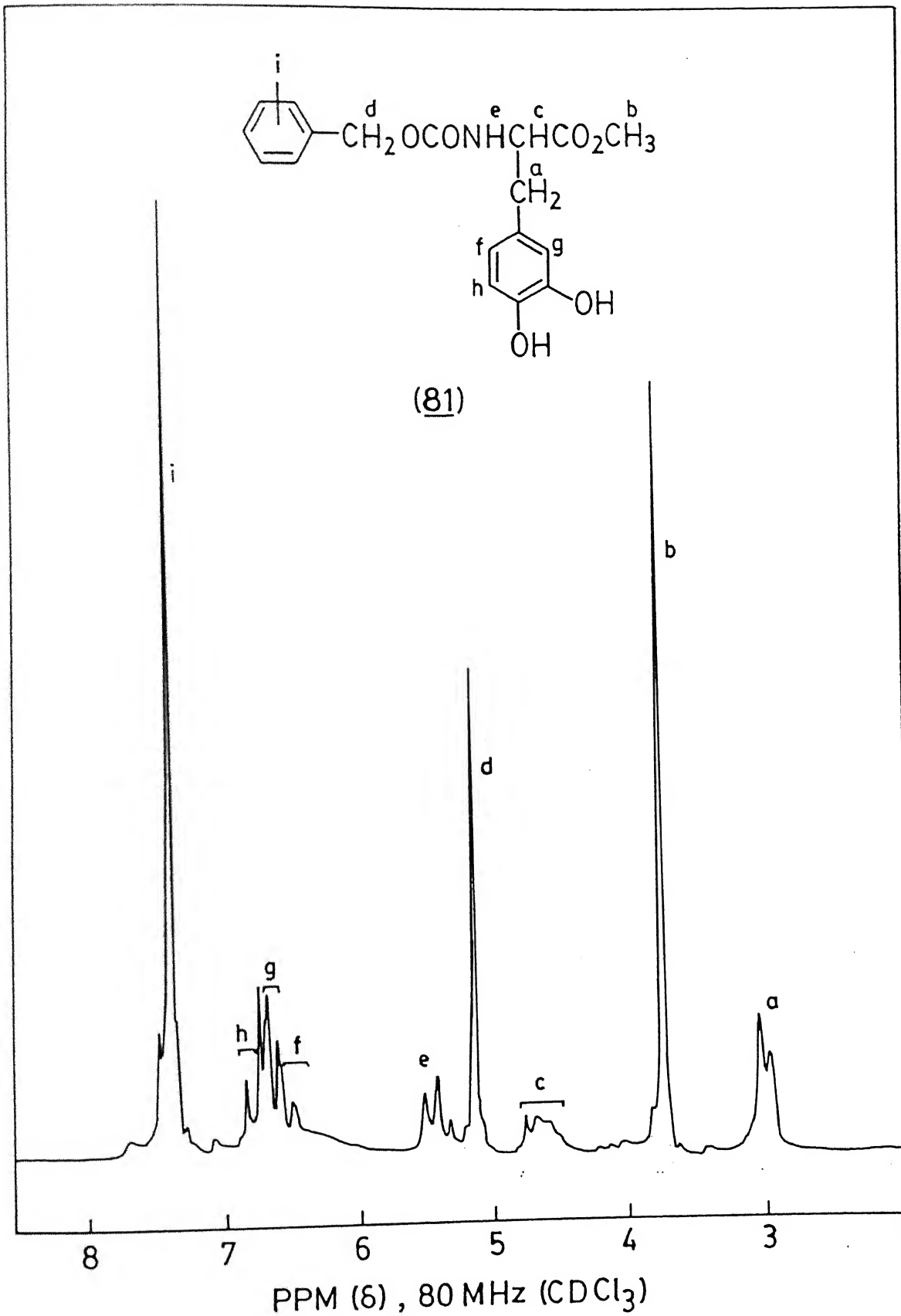
HDO
b
a
c
f
e
d
10
9
8
7
6
5
4
3
PPM (δ), 80 MHz (D2O)


a
CH3
H3C–C–OCONHCHCO2CH3
CH3
e d c
CH2 b
g h
f
NO2
OH
i
(78)
i
h
g
f
e
d
c
b
a
11 10 9 8 7 6 5 4 3 2 1
PPM (δ), 80 MHz (CDCl3)

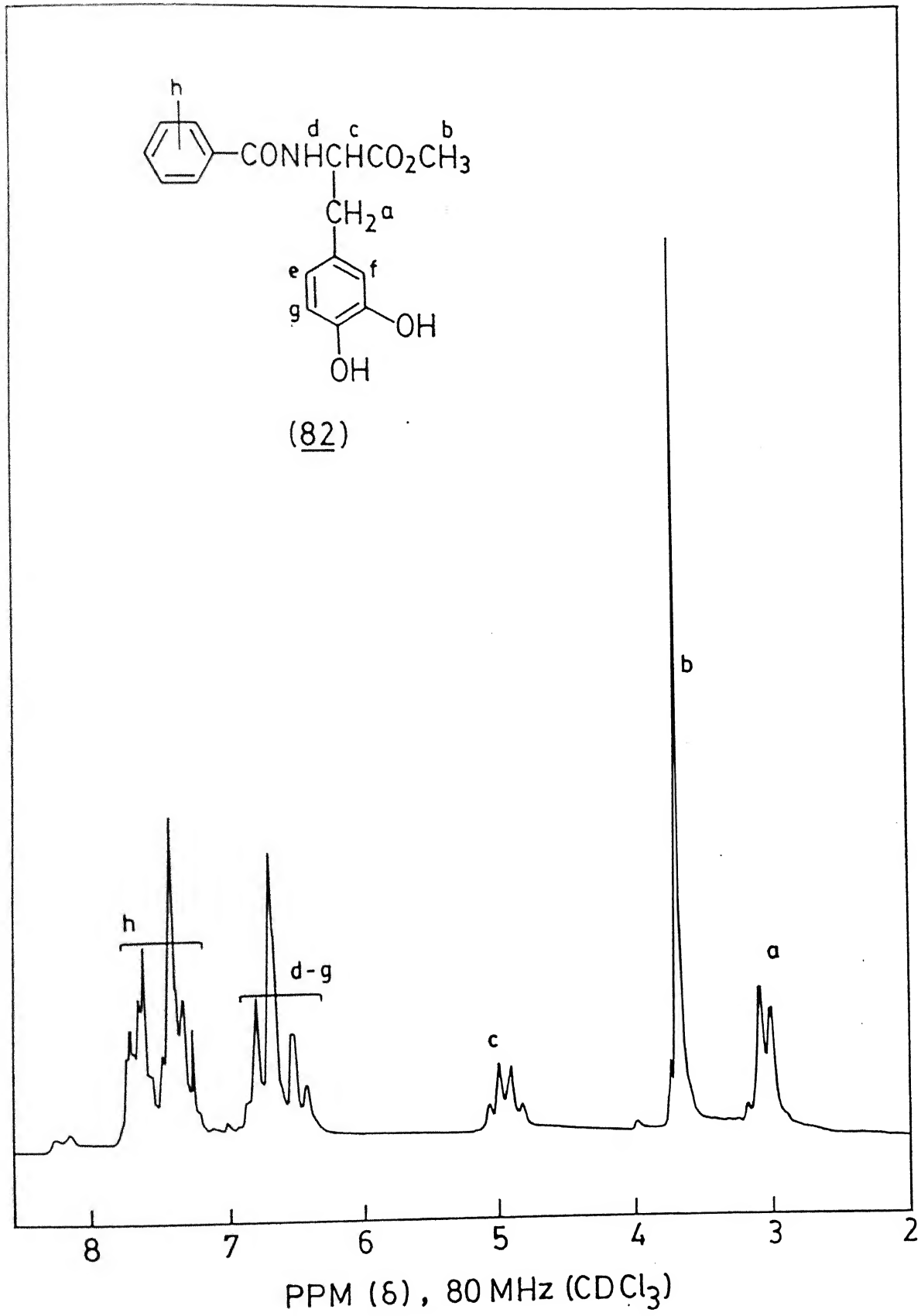


(79)

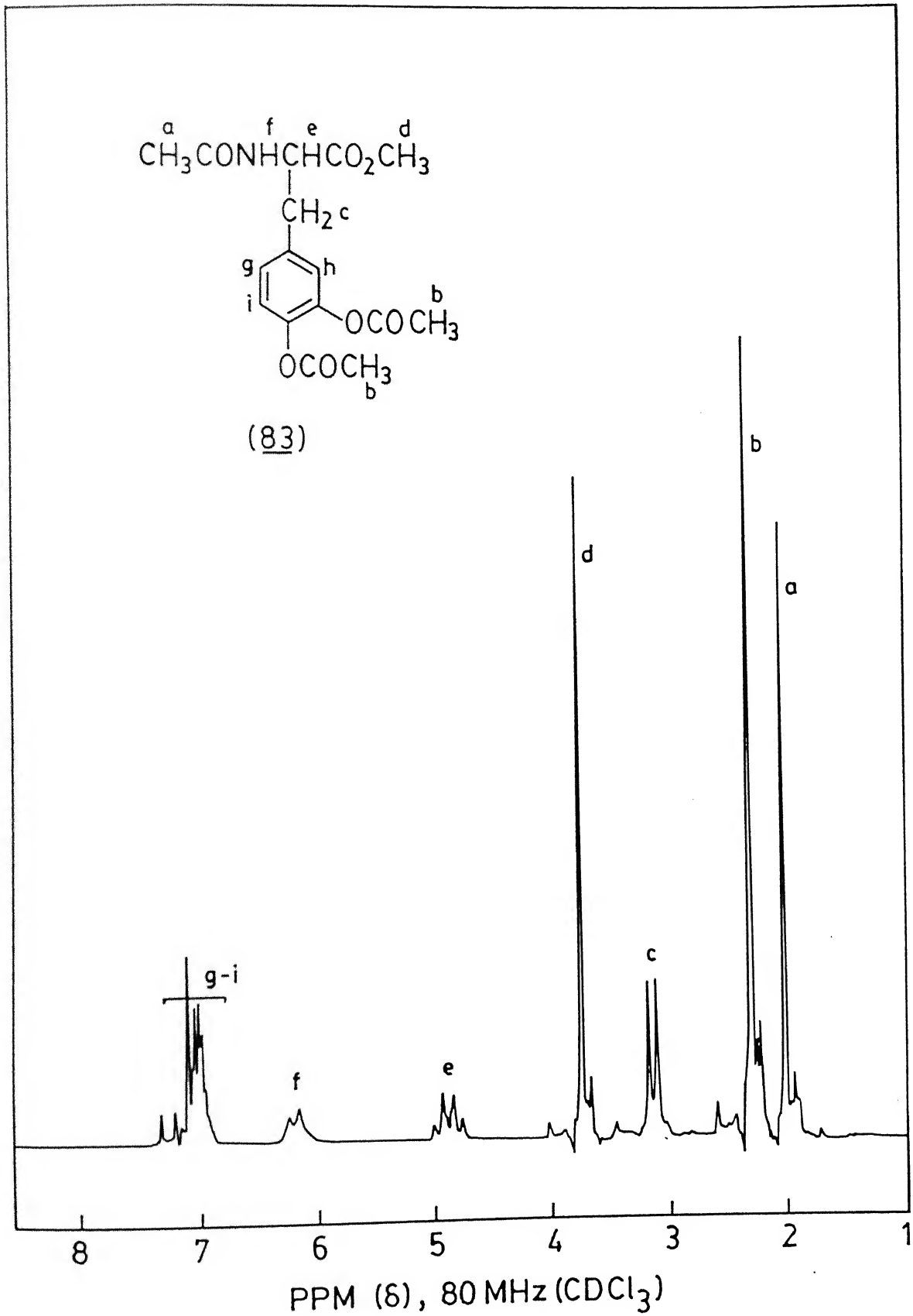

h
g
c
b
HCONHCHCO2CH3
CH2a
d
e
f
OH
OH
(80)
h
e
f
d
CHCl3
g
c
b
a
8
7
6
5
4
3
PPM (δ), 80 MHz (CDCl3 - DMSO - d6)

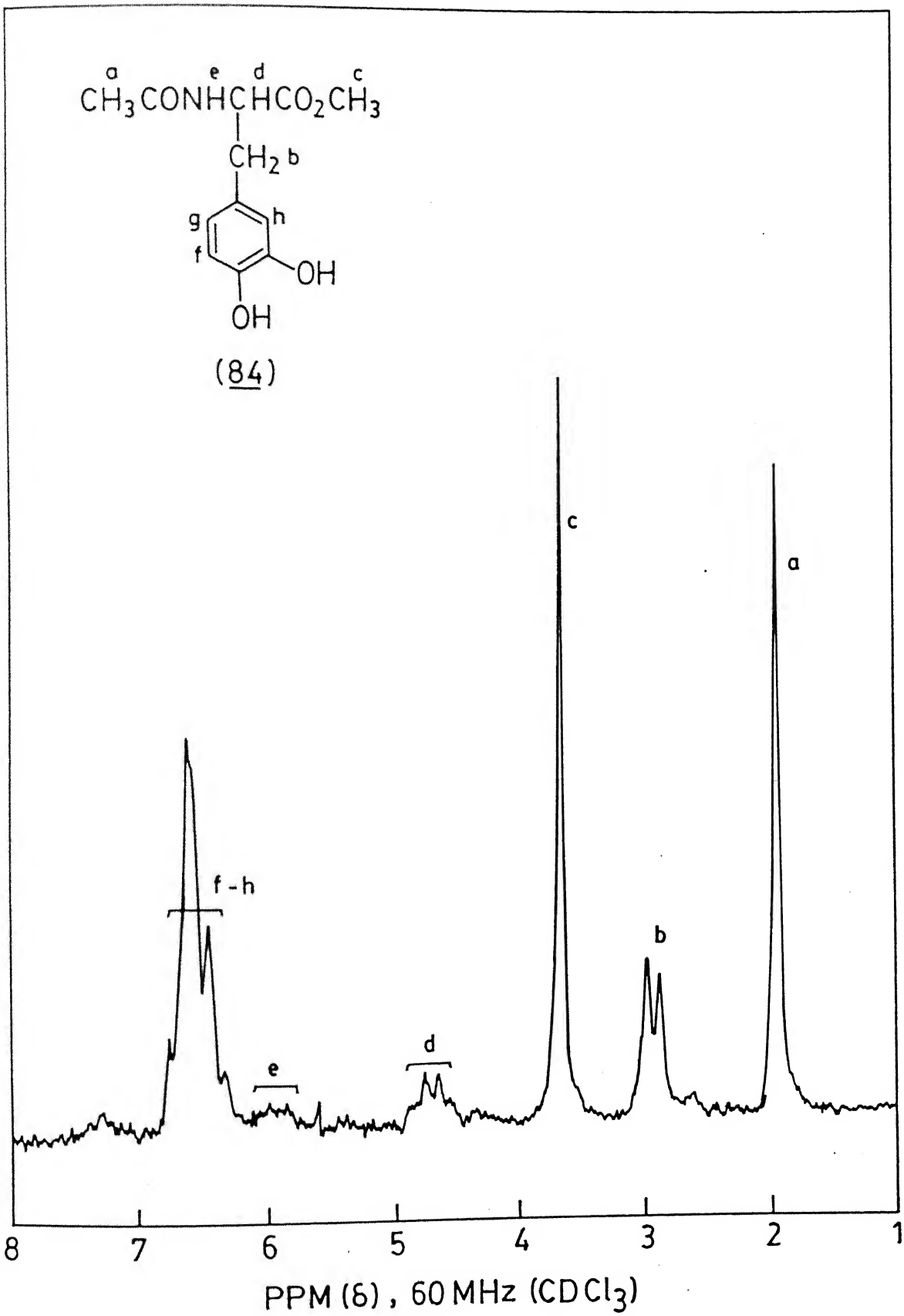

i
d
e
c
b
CH2OCONHCHCO2CH3
a
CH2
f
g
h
OH
OH
i
b
d
g
h
f
e
c
a
8
7
6
5
4
3
2
PPM (δ) , 80 MHz (CDCl3)

(81)

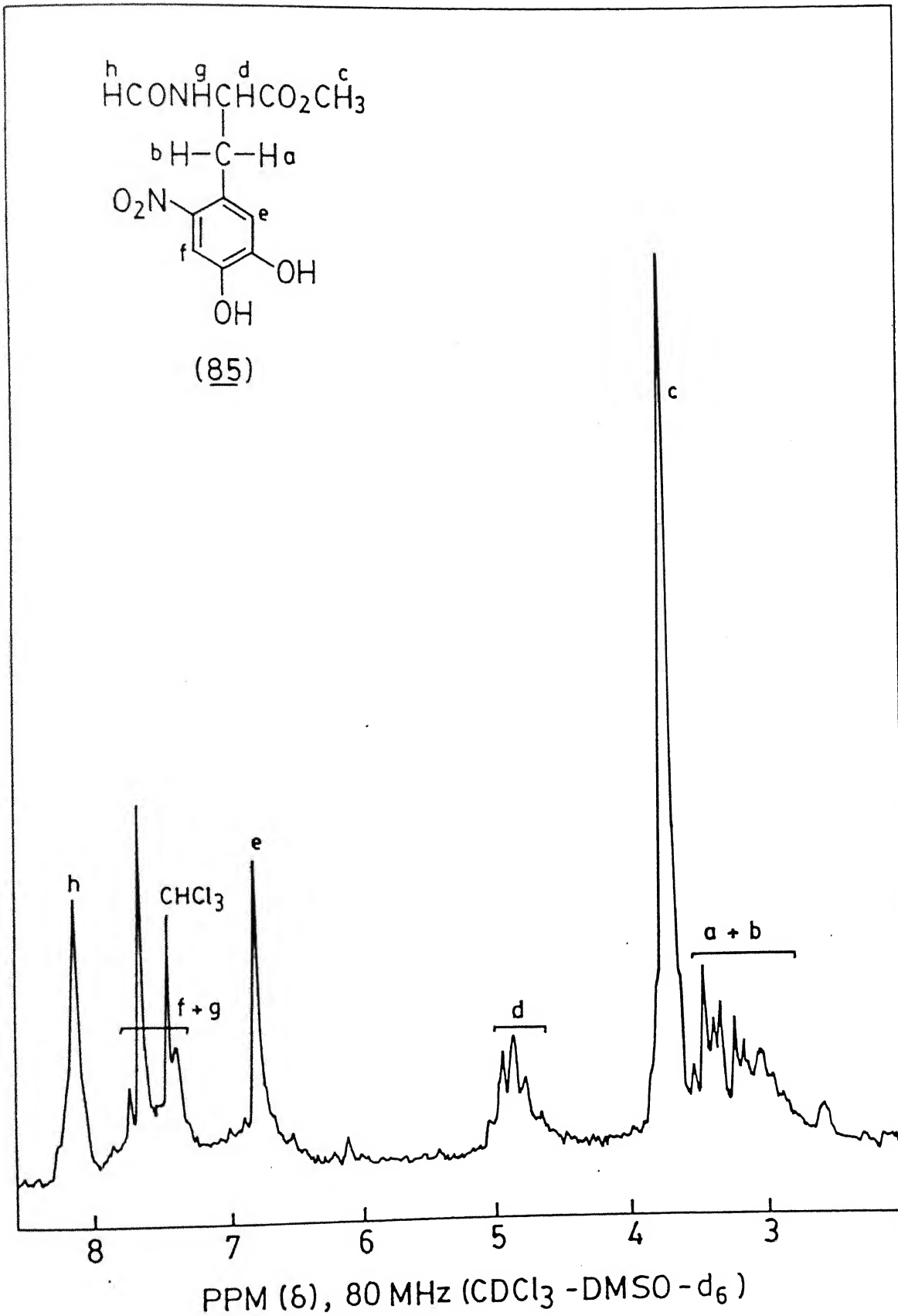

h
d c b
CONHCHCO2CH3
CH2 a
e f
g
OH
OH
h
d-g
c
b
a
8
7
6
5
4
3
2
PPM (δ), 80 MHz (CDCl3)

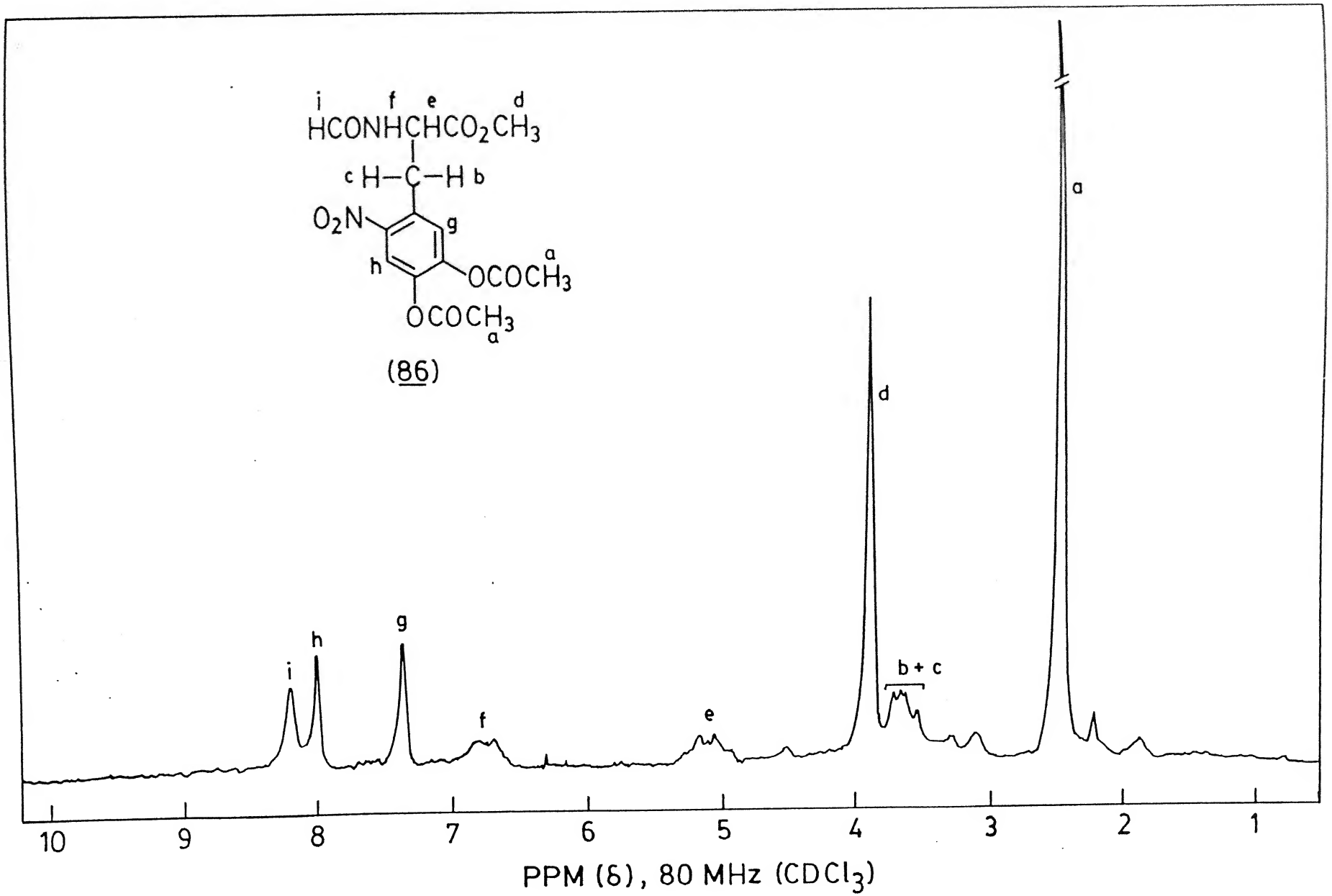
(82)

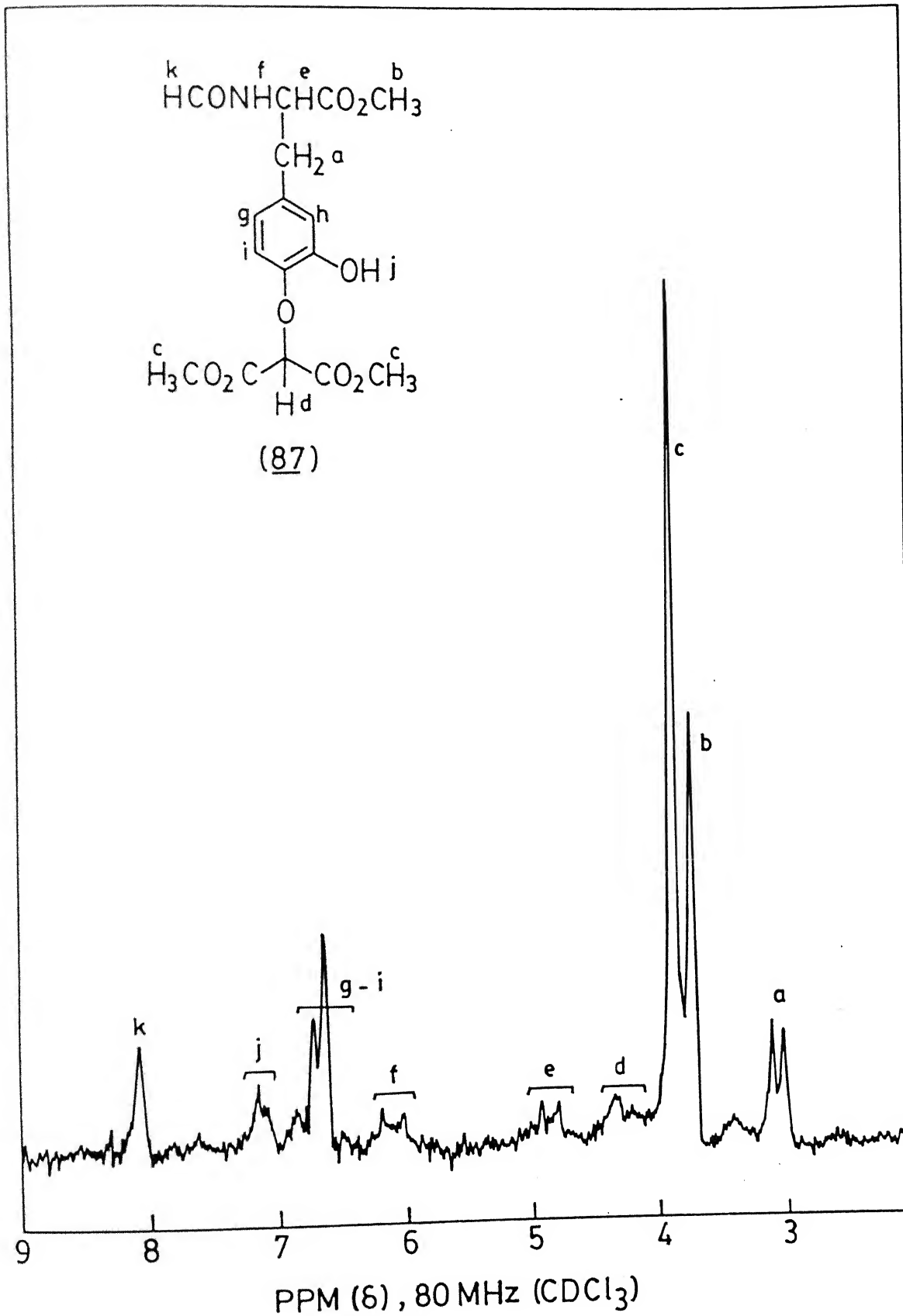

a f e d
CH3CONHCHCO2CH3
CH2 c
g h
i
b
OCOCH3
OCOCH3
b
(83)
b
d
a
c
g-i
f
e
8
7
6
5
4
3
2
1
PPM (δ), 80 MHz (CDCl3)

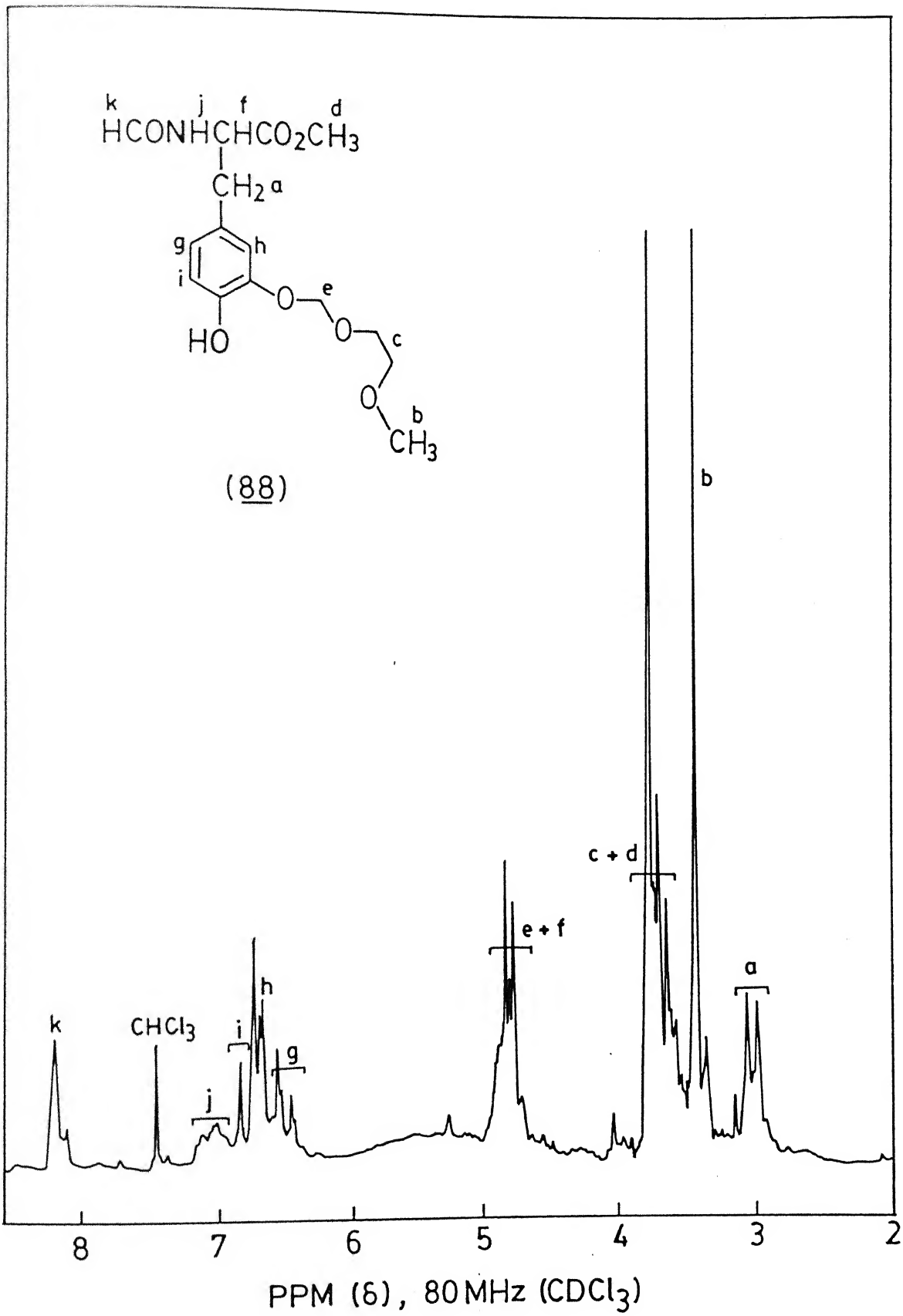

a
e
d
c
CH3CONHCHCO2CH3
CH2 b
g
h
f
OH
OH
f-h
e
d
c
b
a
8
7
6
5
4
3
2
1
PPM (δ), 60 MHz (CDCl3)

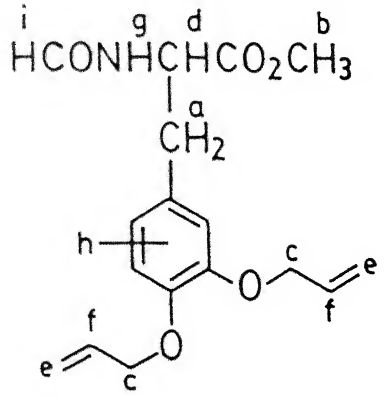
(84)


h g d c
HCONHCHCO2CH3
b H—C—H a
O2N
e
f
OH
OH
(85)
h
CHCl3
e
f + g
d
c
a + b
8
7
6
5
4
3
2
PPM (δ), 80 MHz (CDCl3 - DMSO - d6)

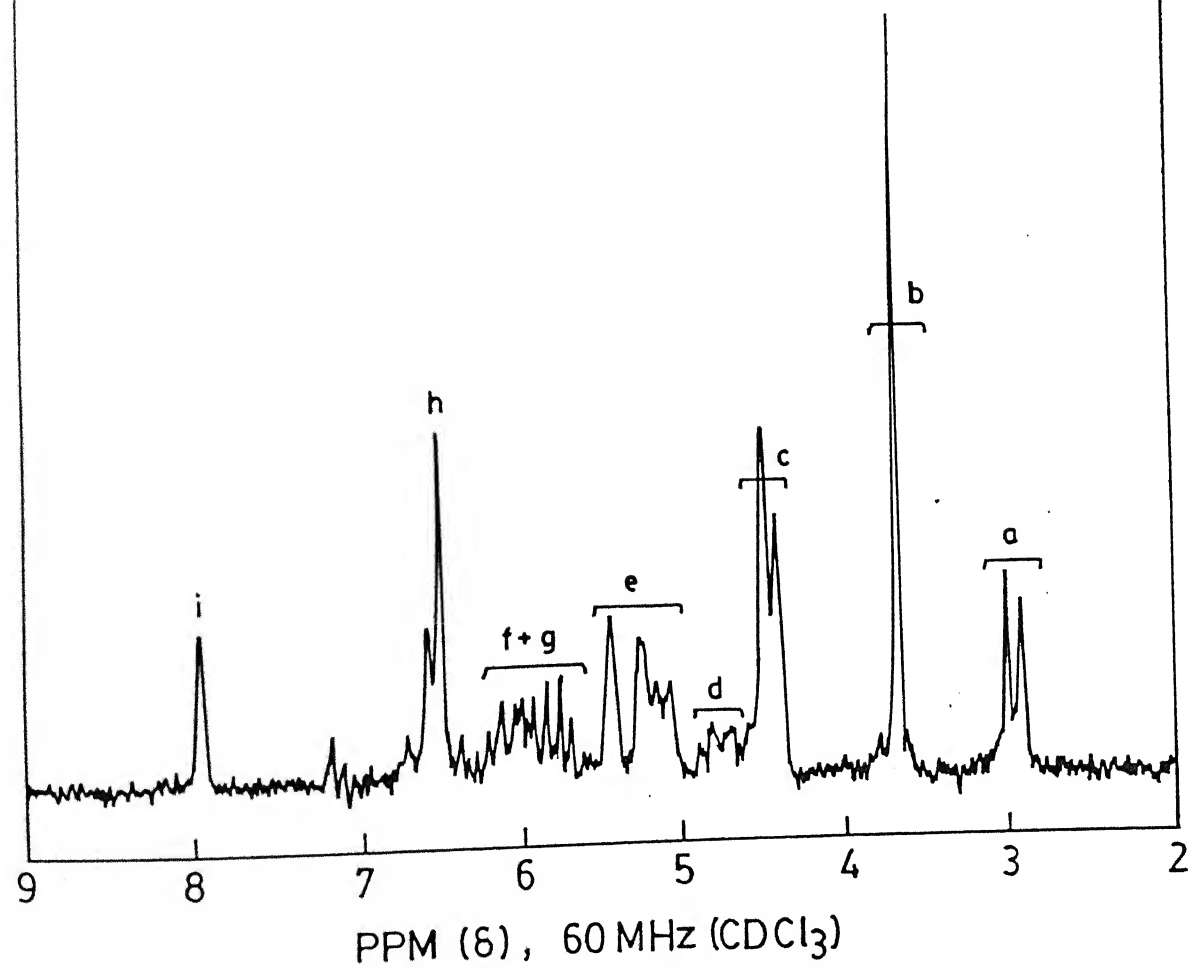

i f e d
HCONHCHCO2CH3
c H—C—H b
O2N
g
h
OCOCH3
a
OCOCH3
a
(86)
a
d
b + c
i
h
g
f
e
10
9
8
7
6
5
4
3
2
1
PPM (δ), 80 MHz (CDCl3)

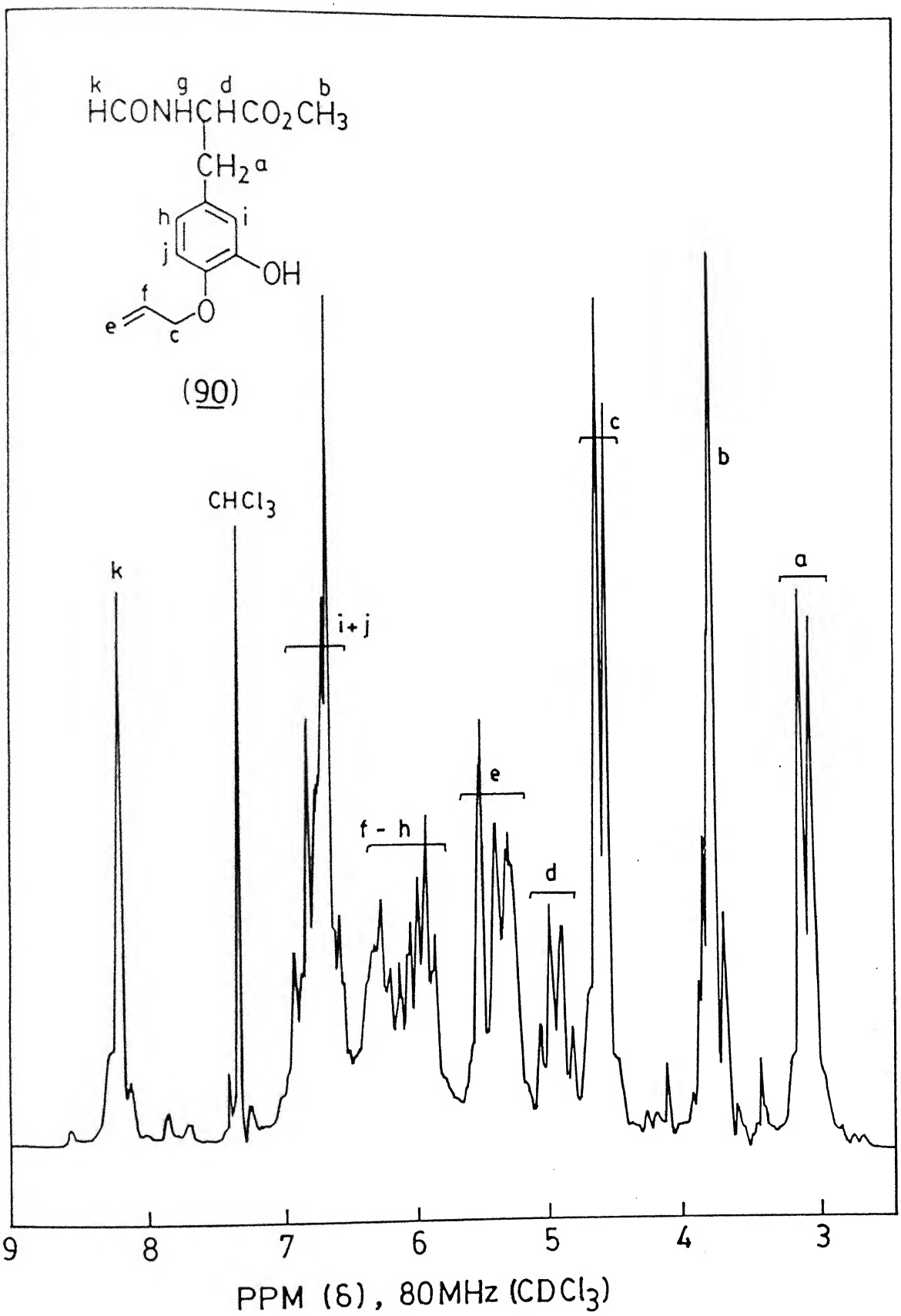

k f e b
HCONHCHCO2CH3
CH2 a
g h
i
OH j
O
c c
H3CO2C CO2CH3
H d
(87)
c
b
a
g - i
k
j
f
e
d
9 8 7 6 5 4 3 2
PPM (δ) , 80 MHz (CDCl3)

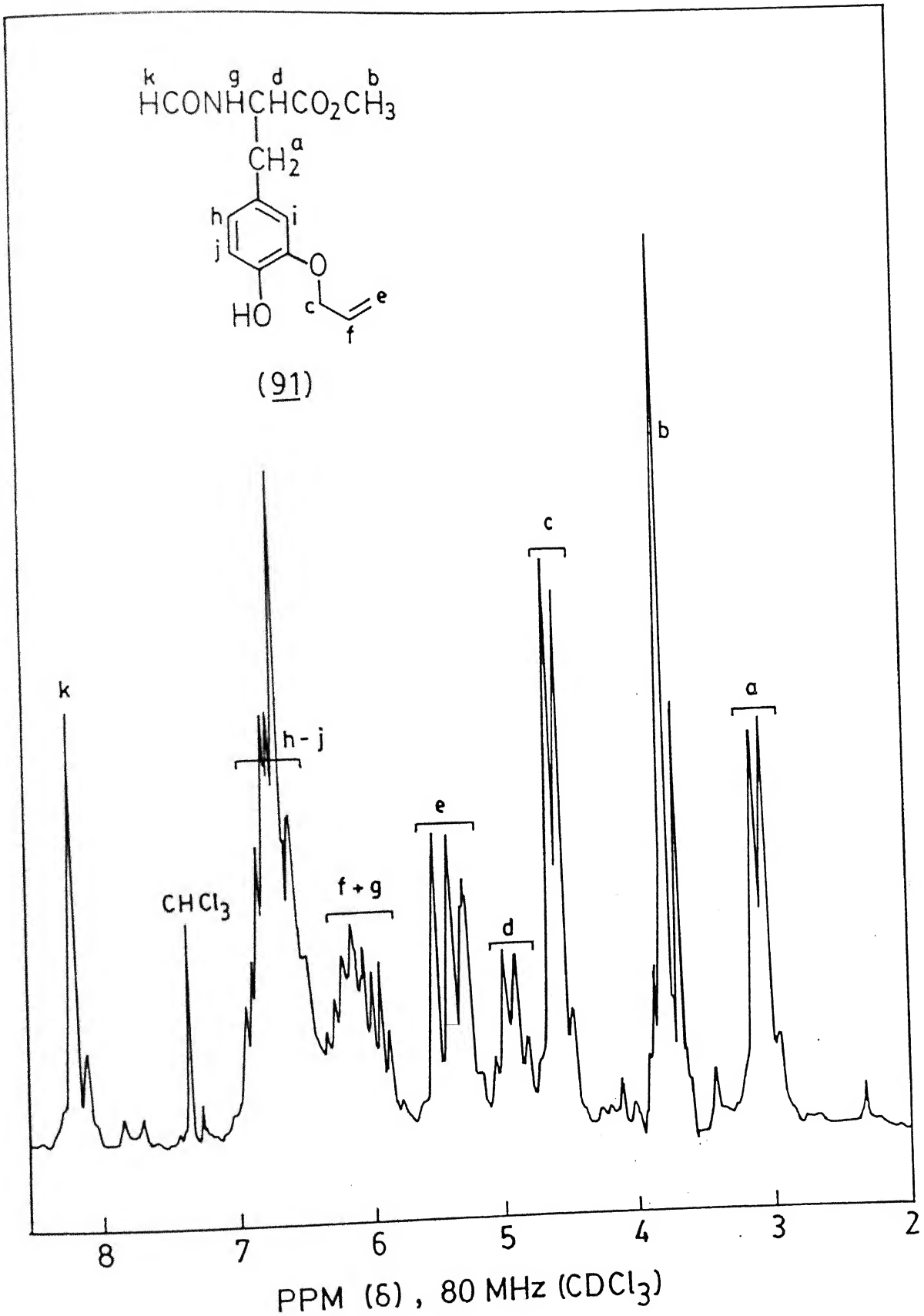

k
j
f
d
HCONHCHCO2CH3
CH2 a
g
h
i
e
O
O
c
HO
O
b
CH3
(88)
b
c + d
e + f
a
k
CHCl3
i
h
g
j
8
7
6
5
4
3
2
PPM (δ), 80 MHz (CDCl3)

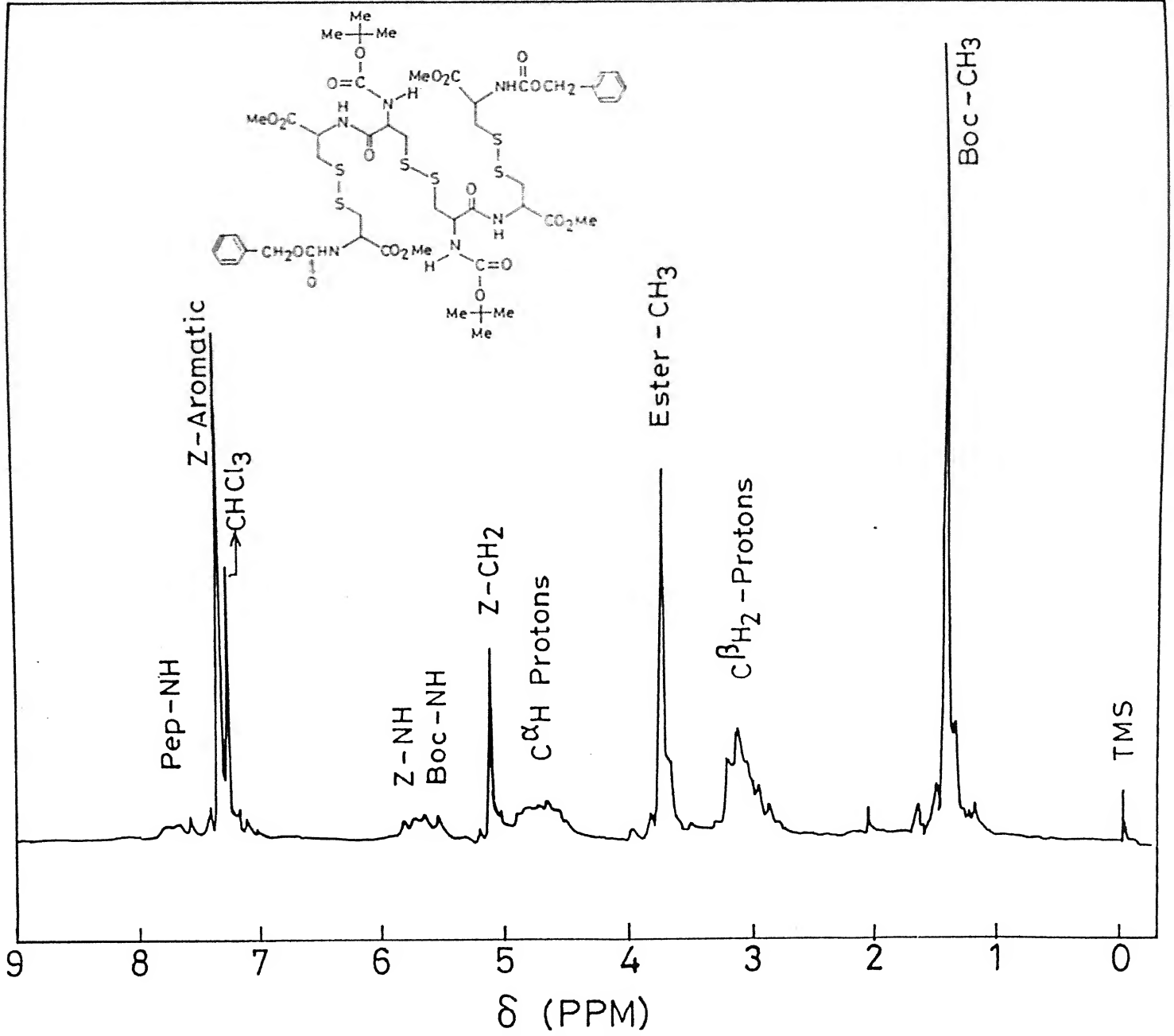

i
g
d
b
HCONHCHCO2CH3
a
CH2
h
c
e
O
f
f
e
O
c

(89)

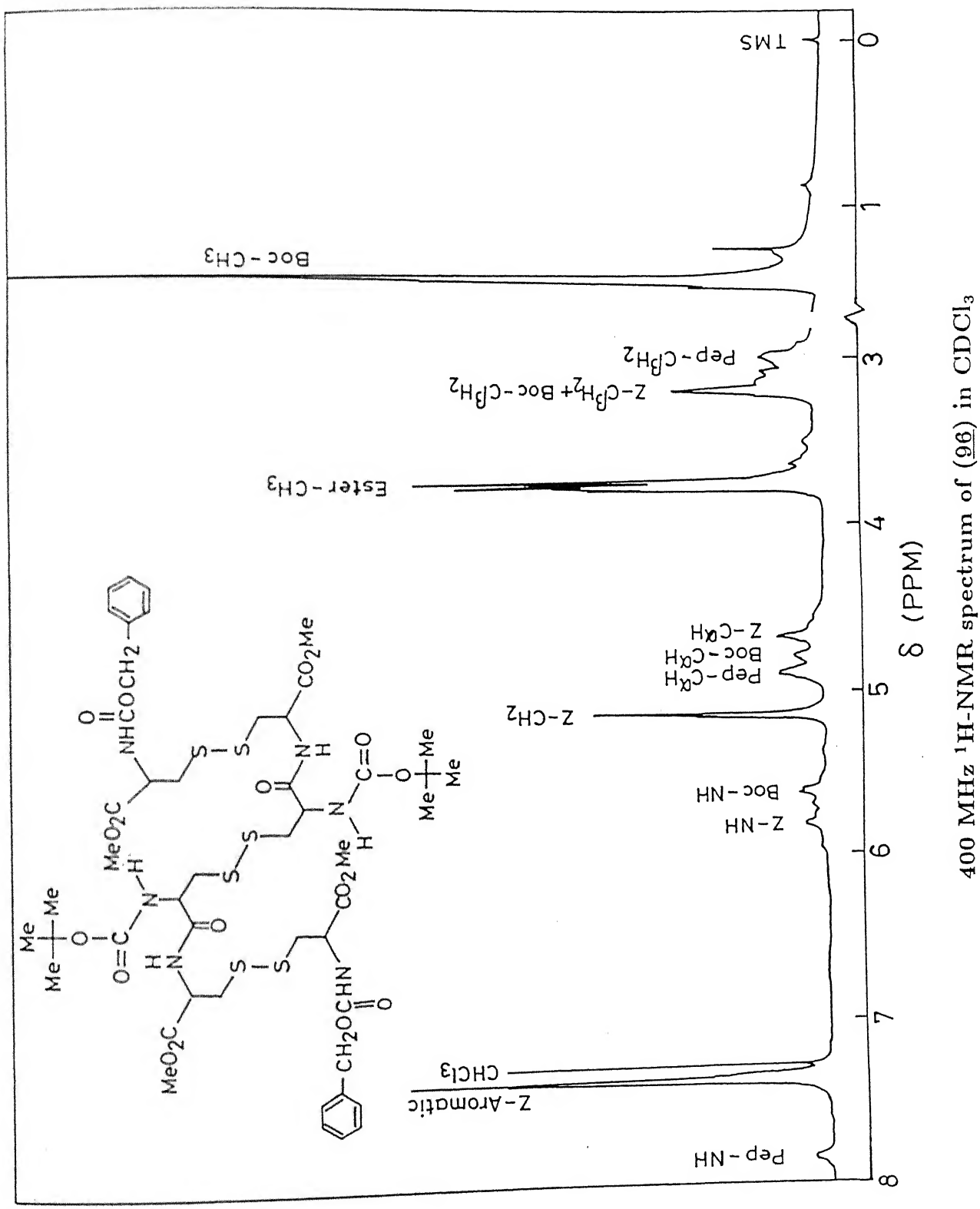

i
h
f + g
e
d
c
b
a
9
8
7
6
5
4
3
2
PPM (δ), 60 MHz (CDCl3)


k g d b
HCONHCHCO2CH3
CH2 a
h i
j
OH
f
e
O
c
(90)
CHCl3
k
i + j
f - h
e
d
c
b
a
9
8
7
6
5
4
3
PPM (δ), 80MHz (CDCl3)

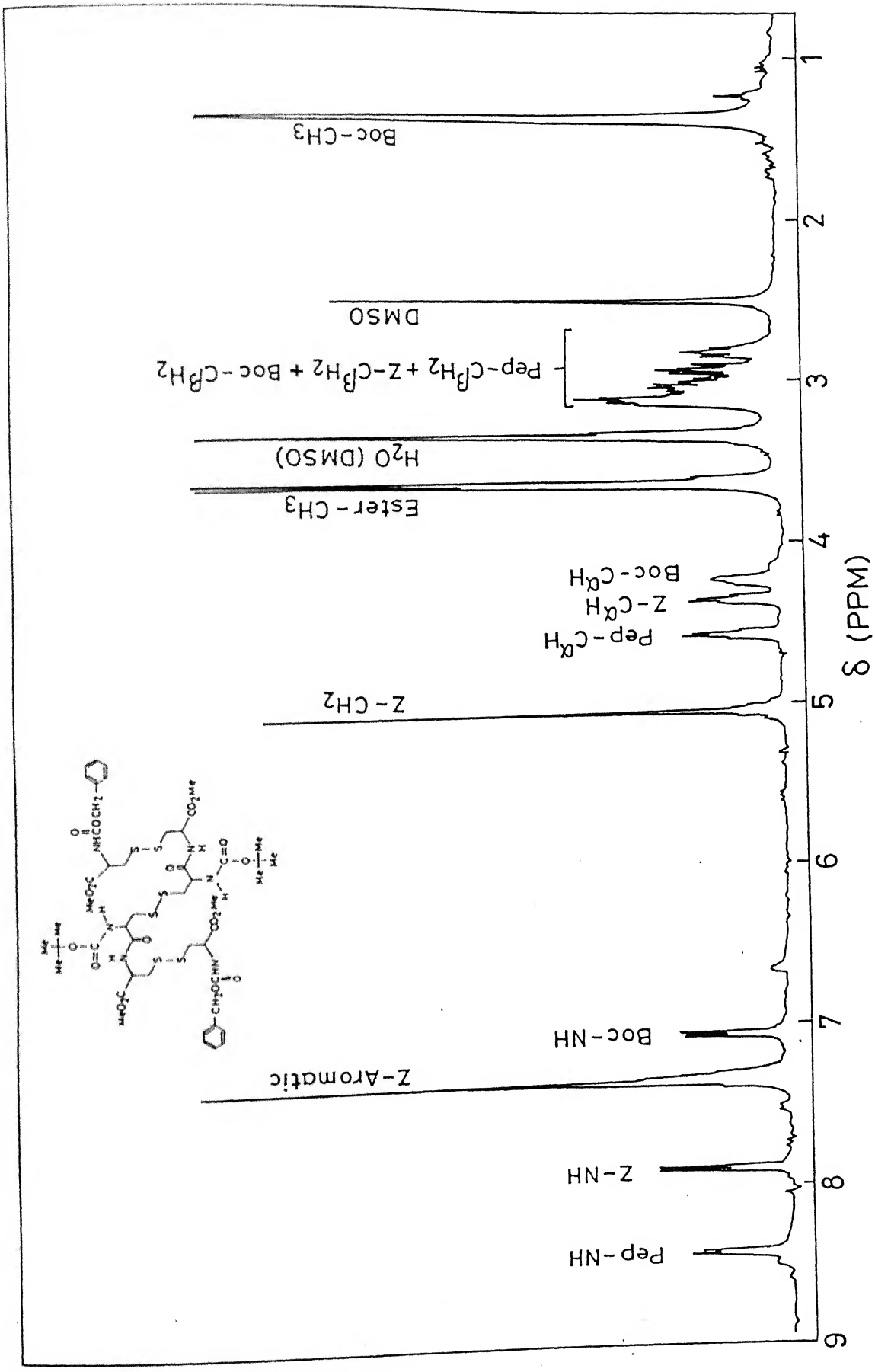

k g d b
HCONHCHCO2CH3
CH2 a
h i
j
O
HO
c
e
f
(91)
k
h - j
CHCl3
f + g
e
d
c
b
a
8
7
6
5
4
3
2
PPM (δ) , 80 MHz (CDCl3)



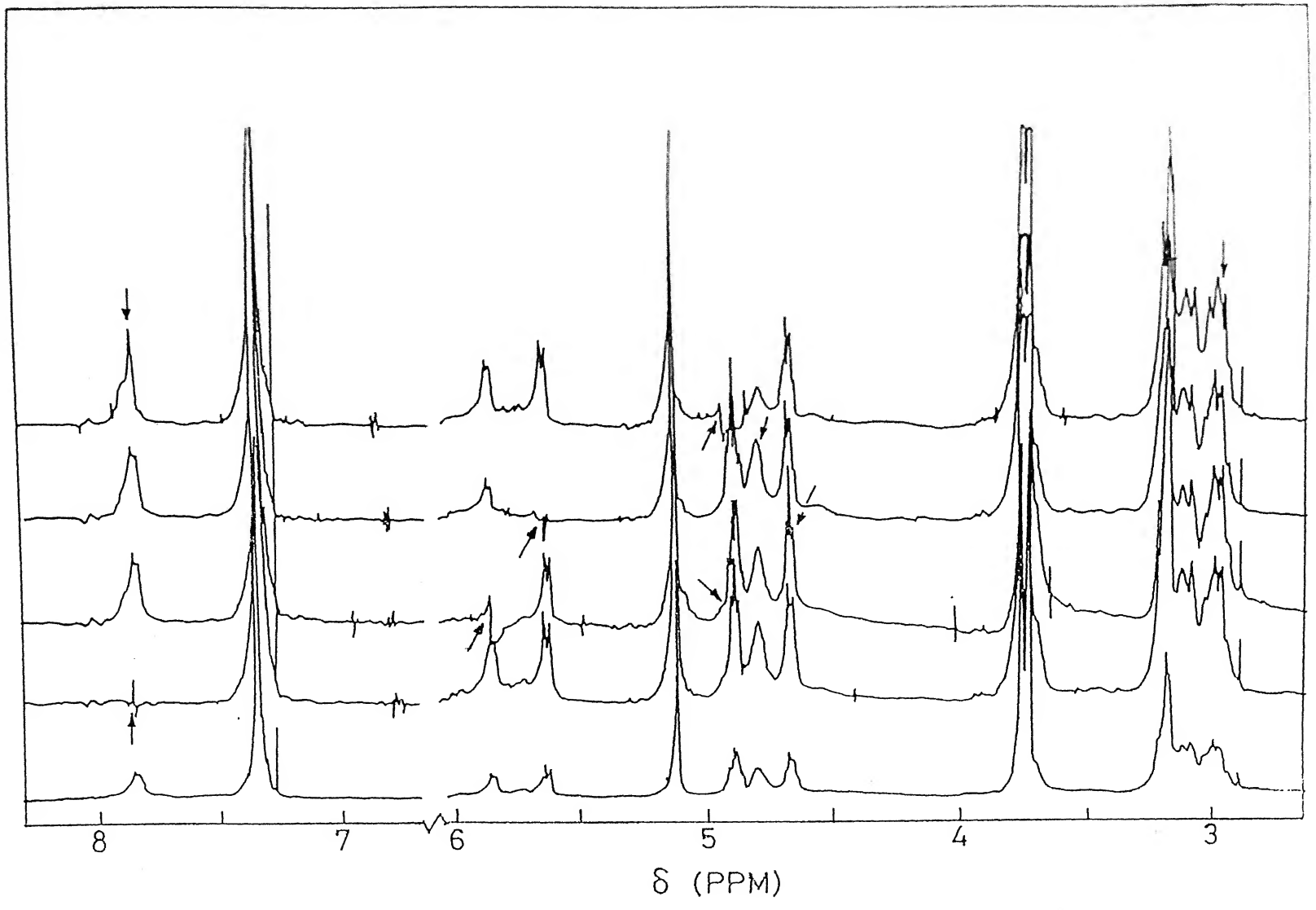
80 MHz ^{1}H-NMR spectrum of (96) in $CDCl_3$



400 MHz ^{1}H-NMR spectrum of (96) in $CDCl_3$

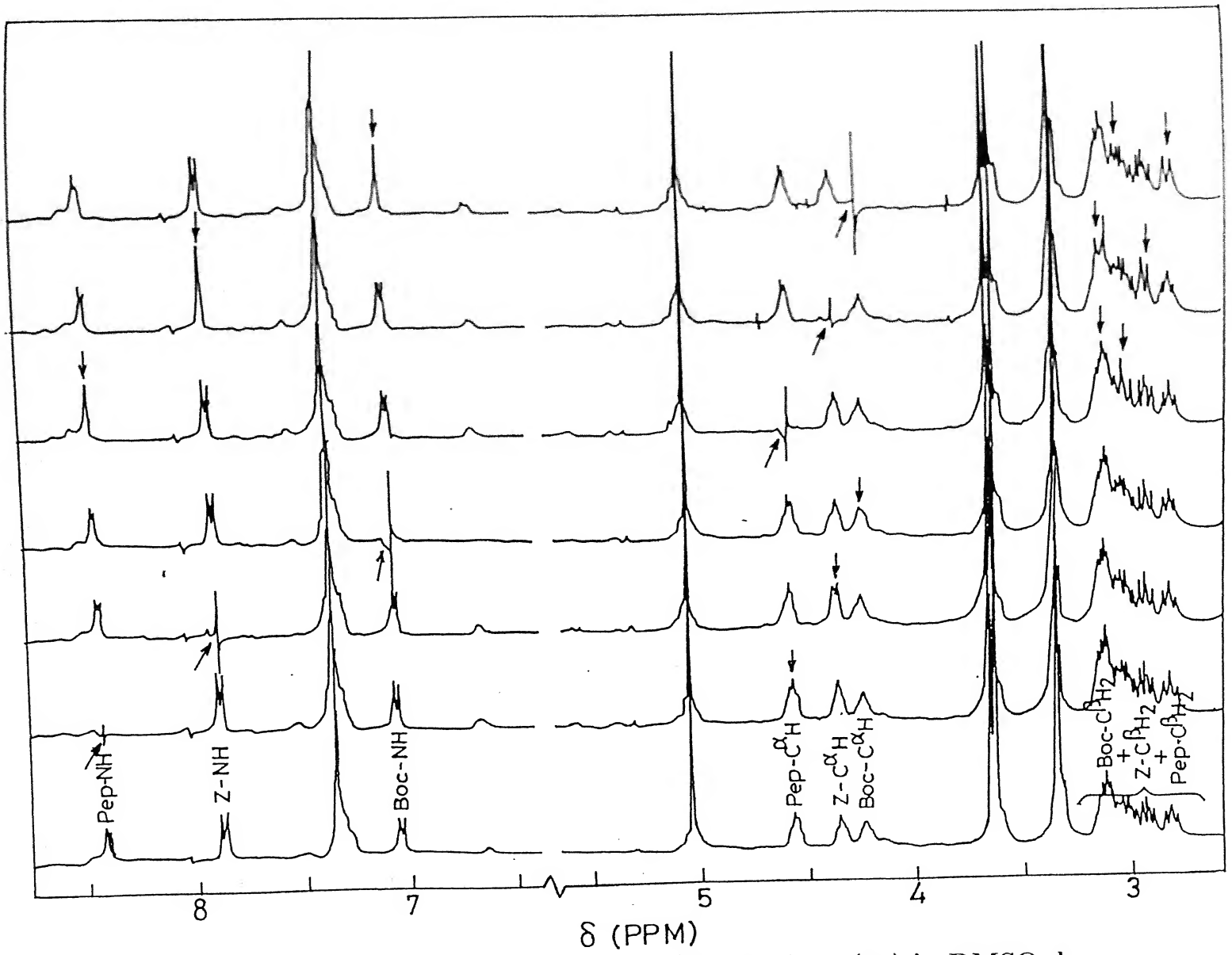


400 MHz ^{1}H-NMR spectrum of (96) in DMSO-d_6



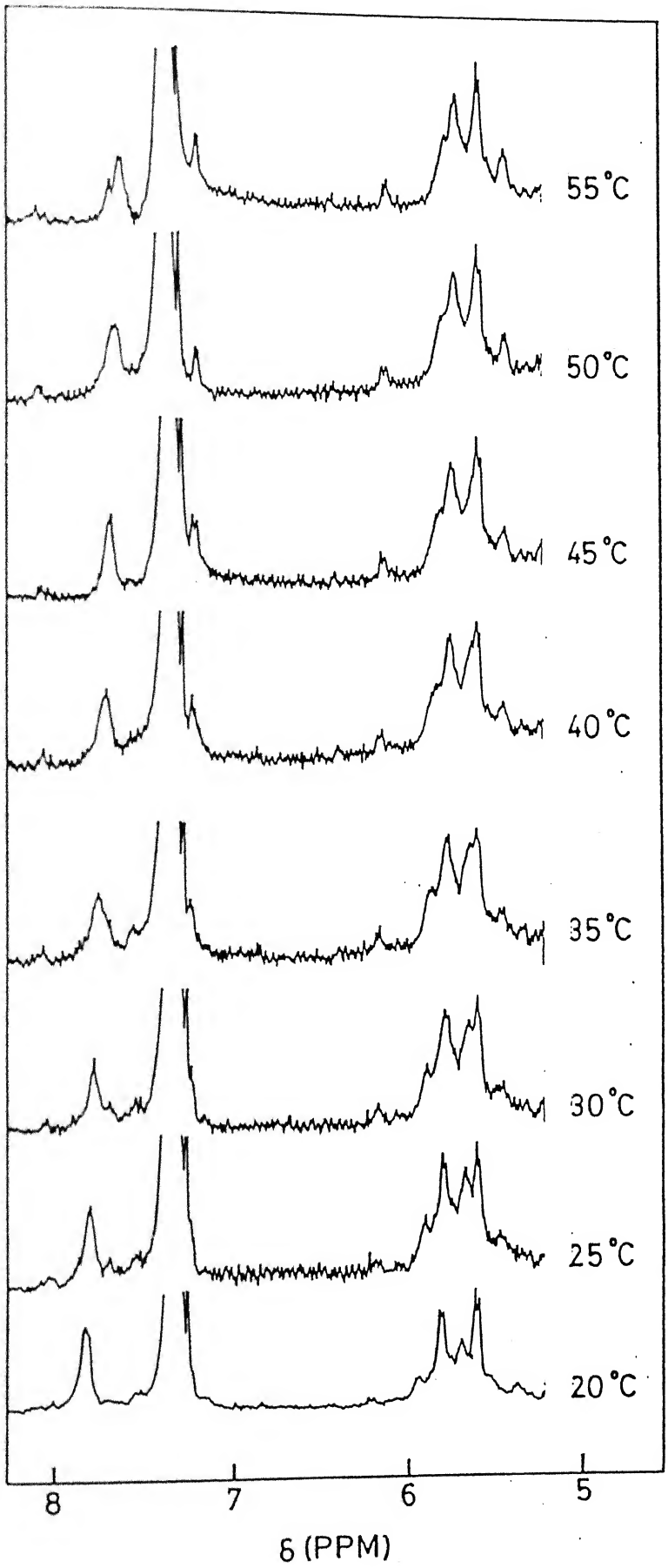
Spin Decoupling Experiments (400 MHz) on (96) in $CDCl_3$

Upward arrows indicate the irradiated protons and downward arrows indicate the observed protons

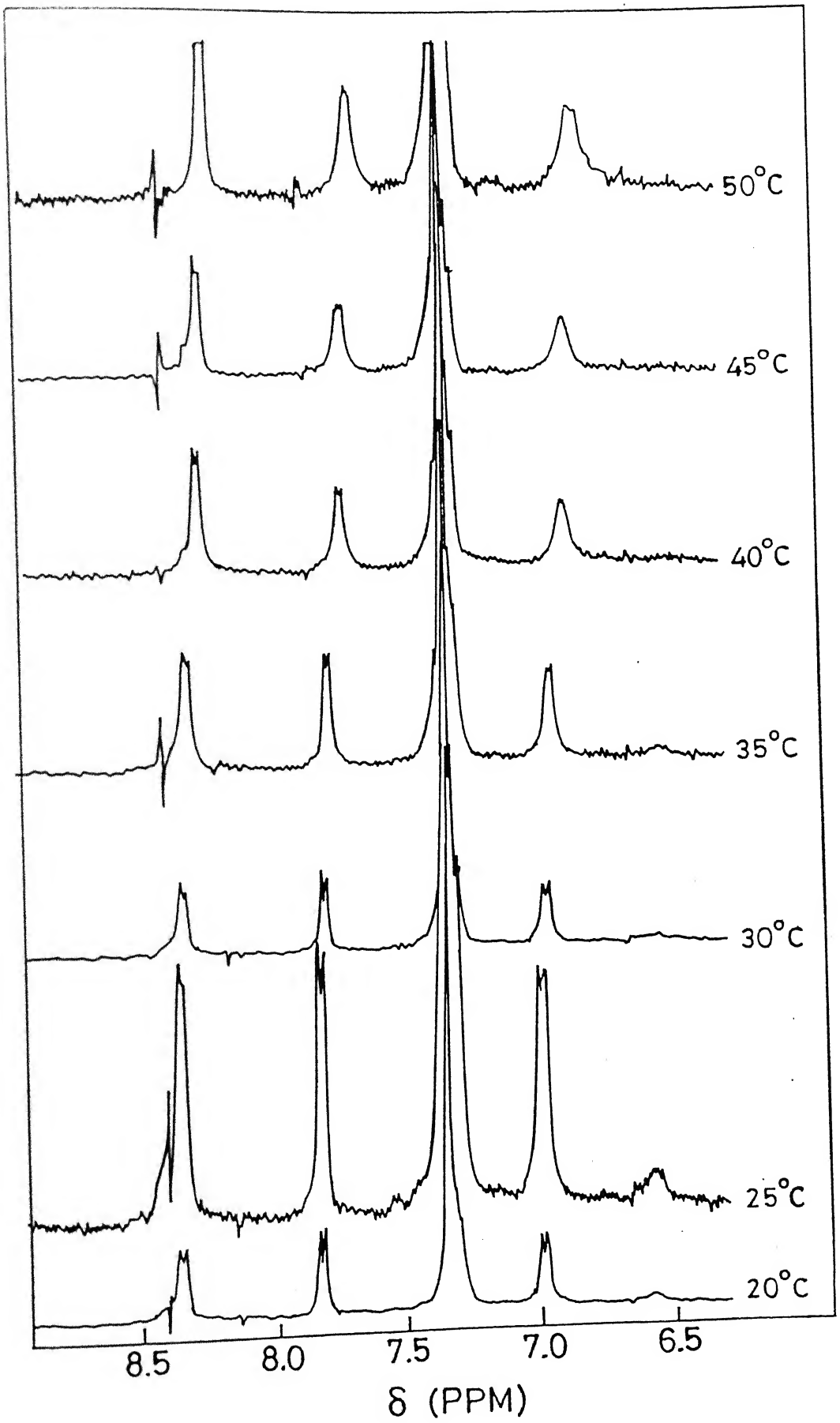


Spin Decoupling Experiments (400 MHz) on (96) in DMSO-d_6

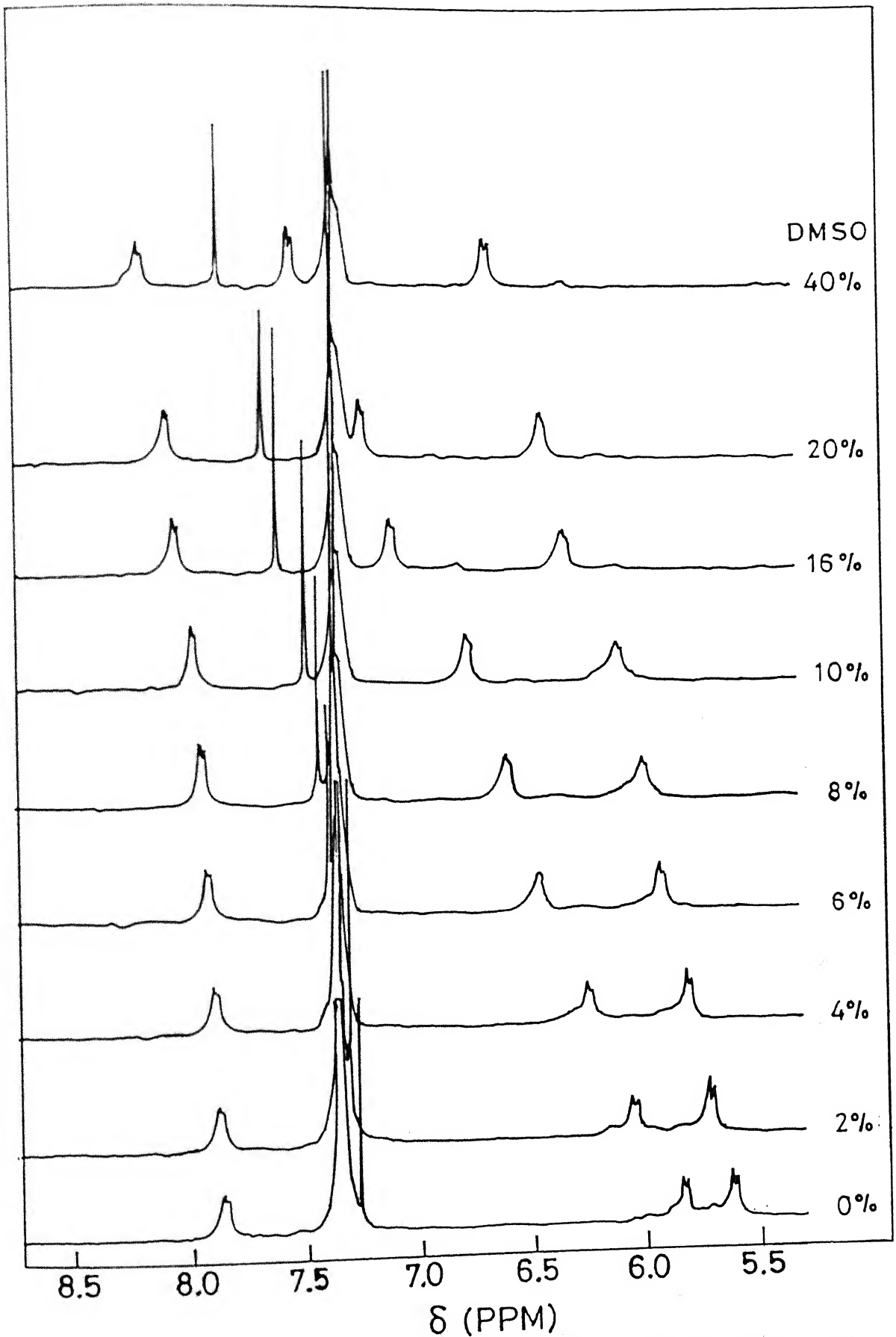
Upward arrows indicate the irradiated protons and downward arrows indicate the observed protons



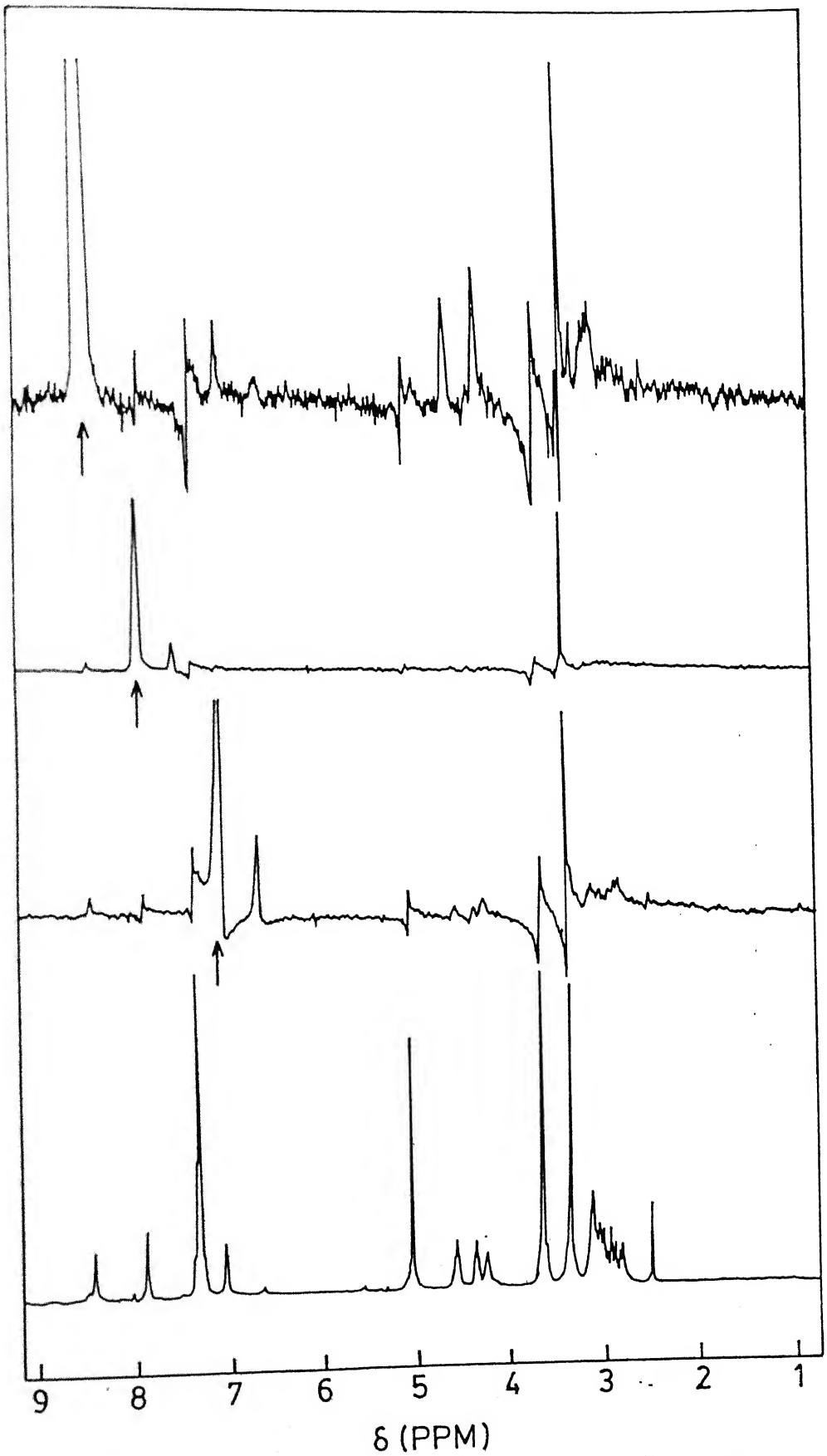
Variable Temperature ^{1}H-NMR (400 MHz) studies on (96) in $CDCl_3$



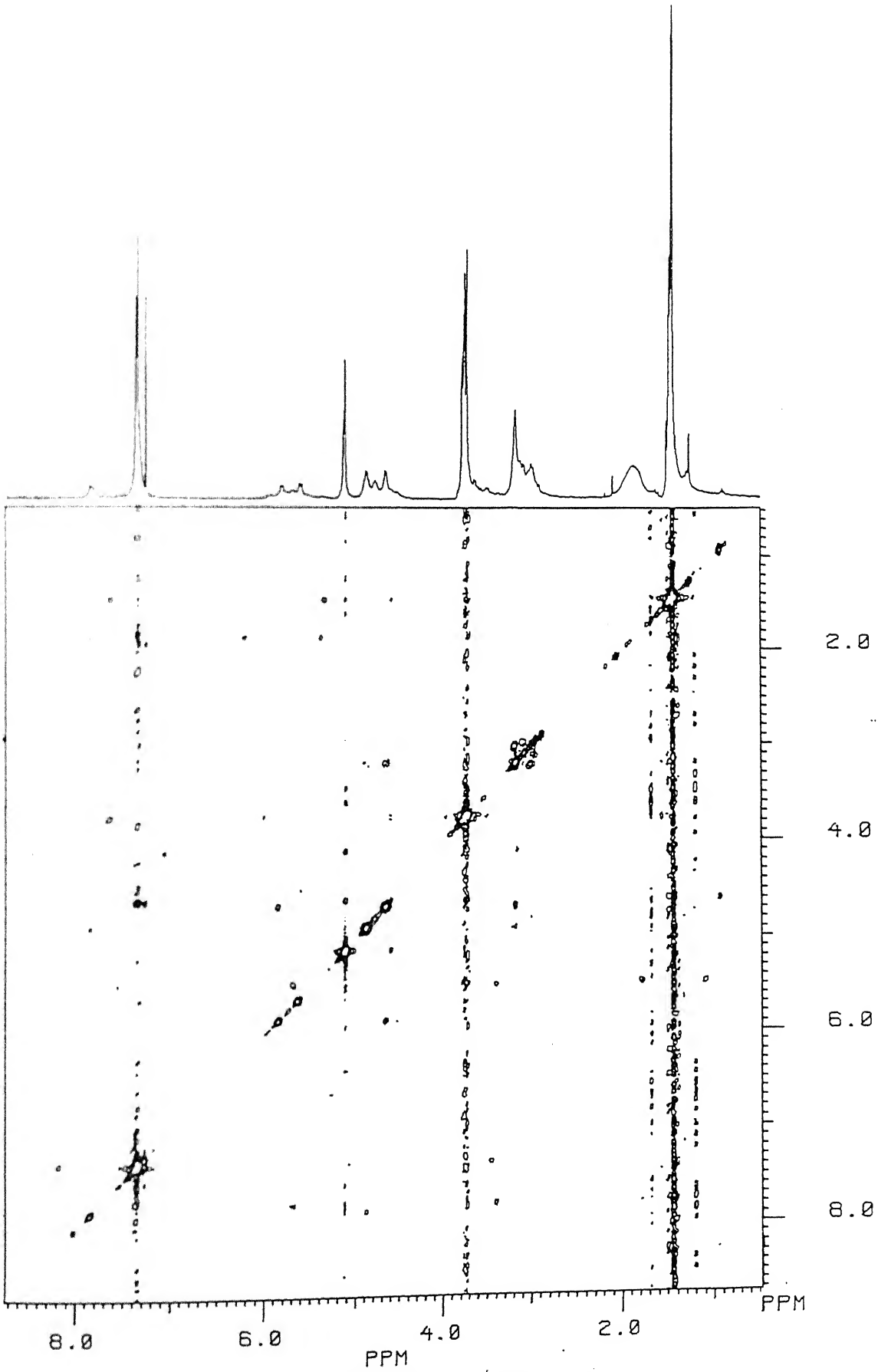
Variable Temperature ^{1}H-NMR (400 MHz) studies on (96) in DMSO-d_6



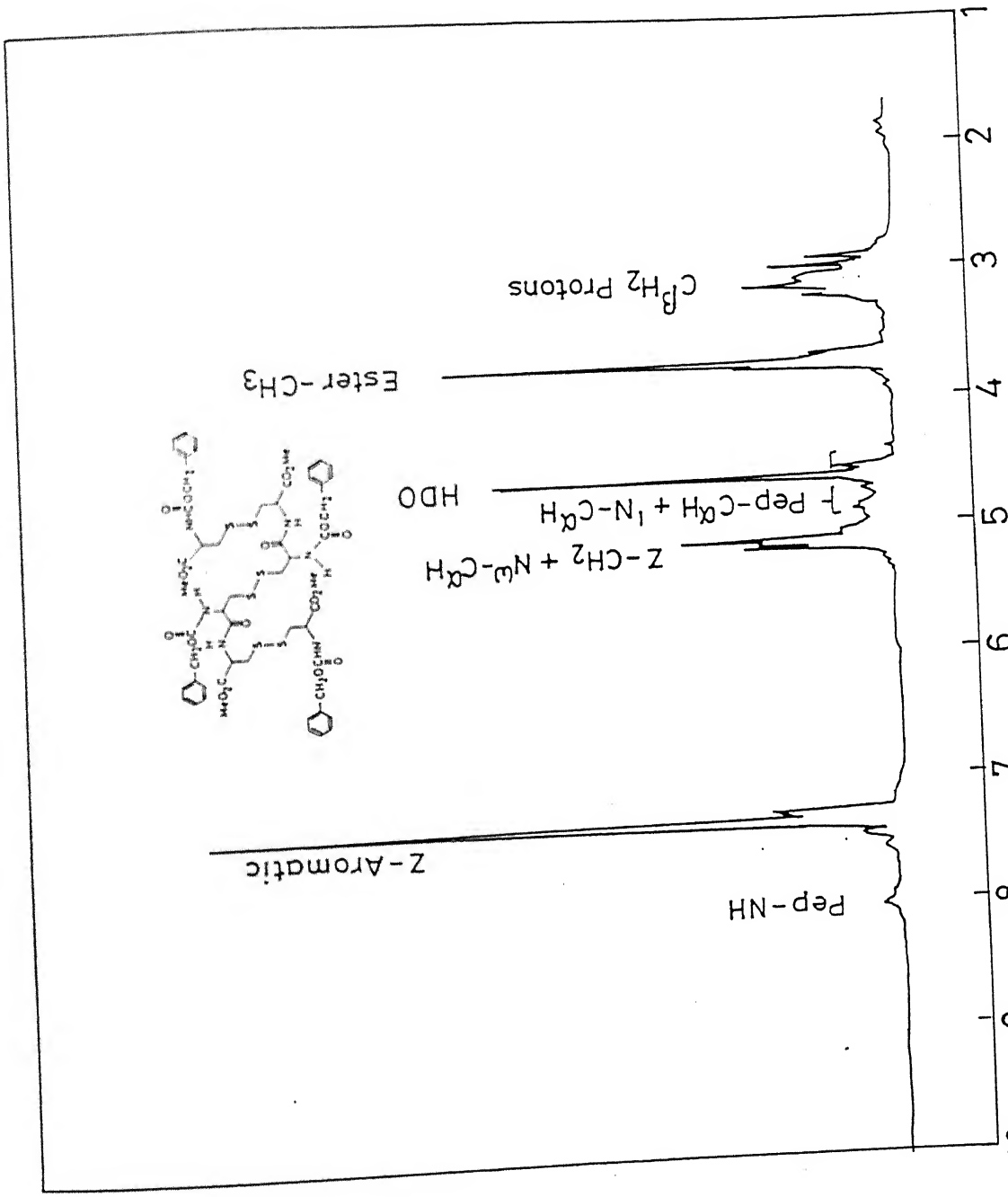
Solvent Dependence-NMR studies (400 MHz) on (96) in $CDCl_3$ and DMSO-d_6



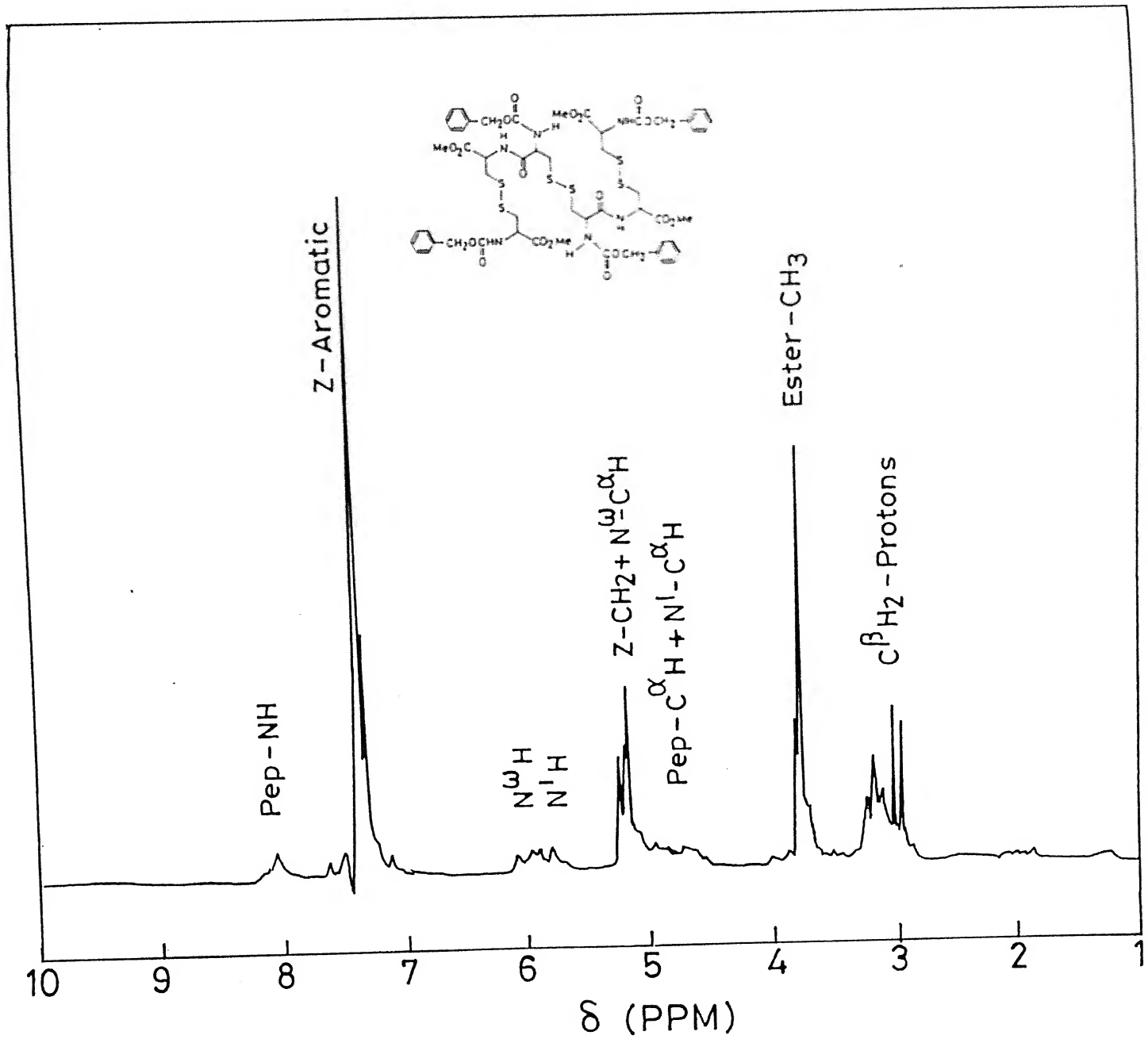
Difference NOE spectra (400 MHz) of (96) in DMSO-d_6



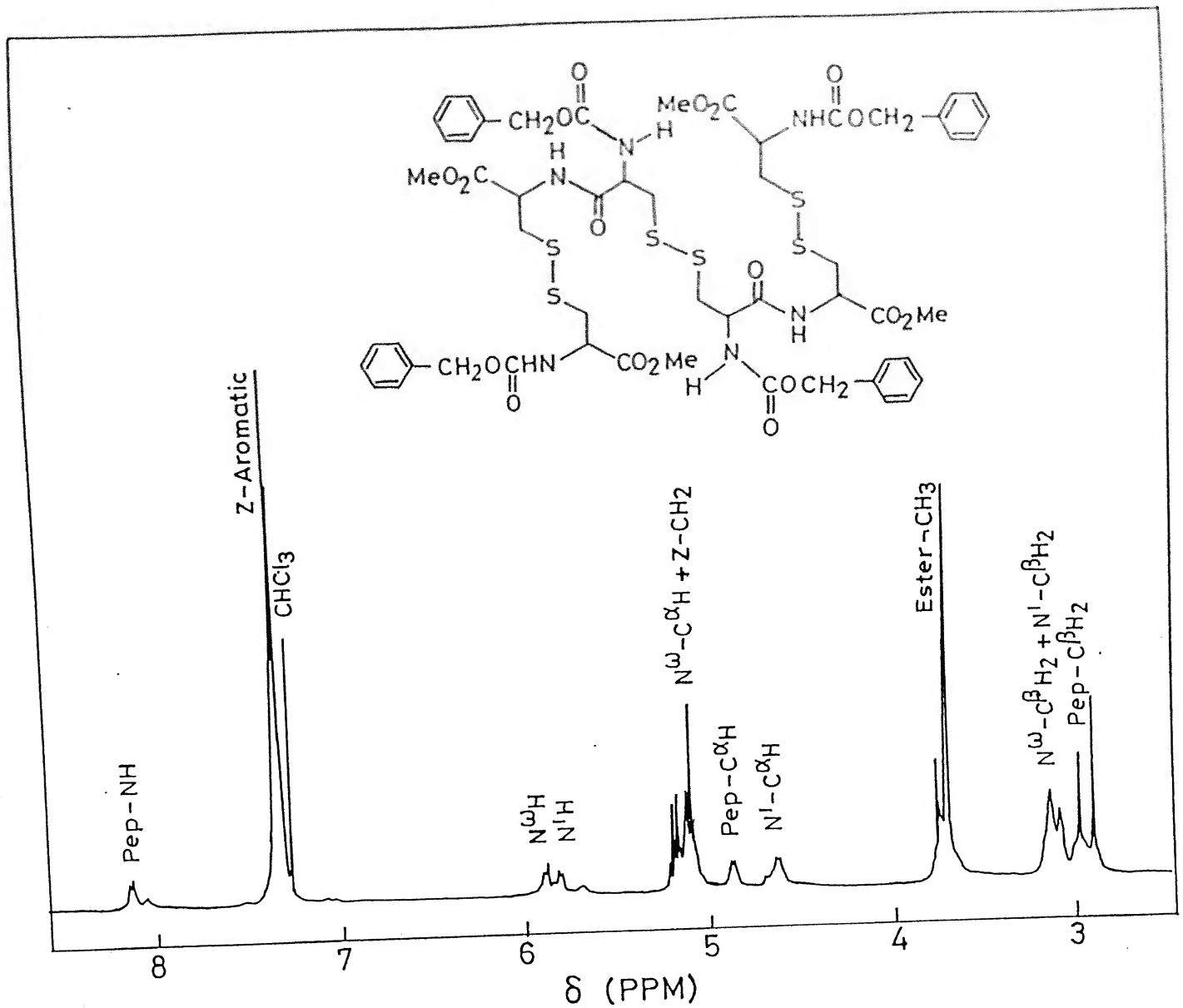
NOESY spectrum (400 MHz) of (96) in $CDCl_3$



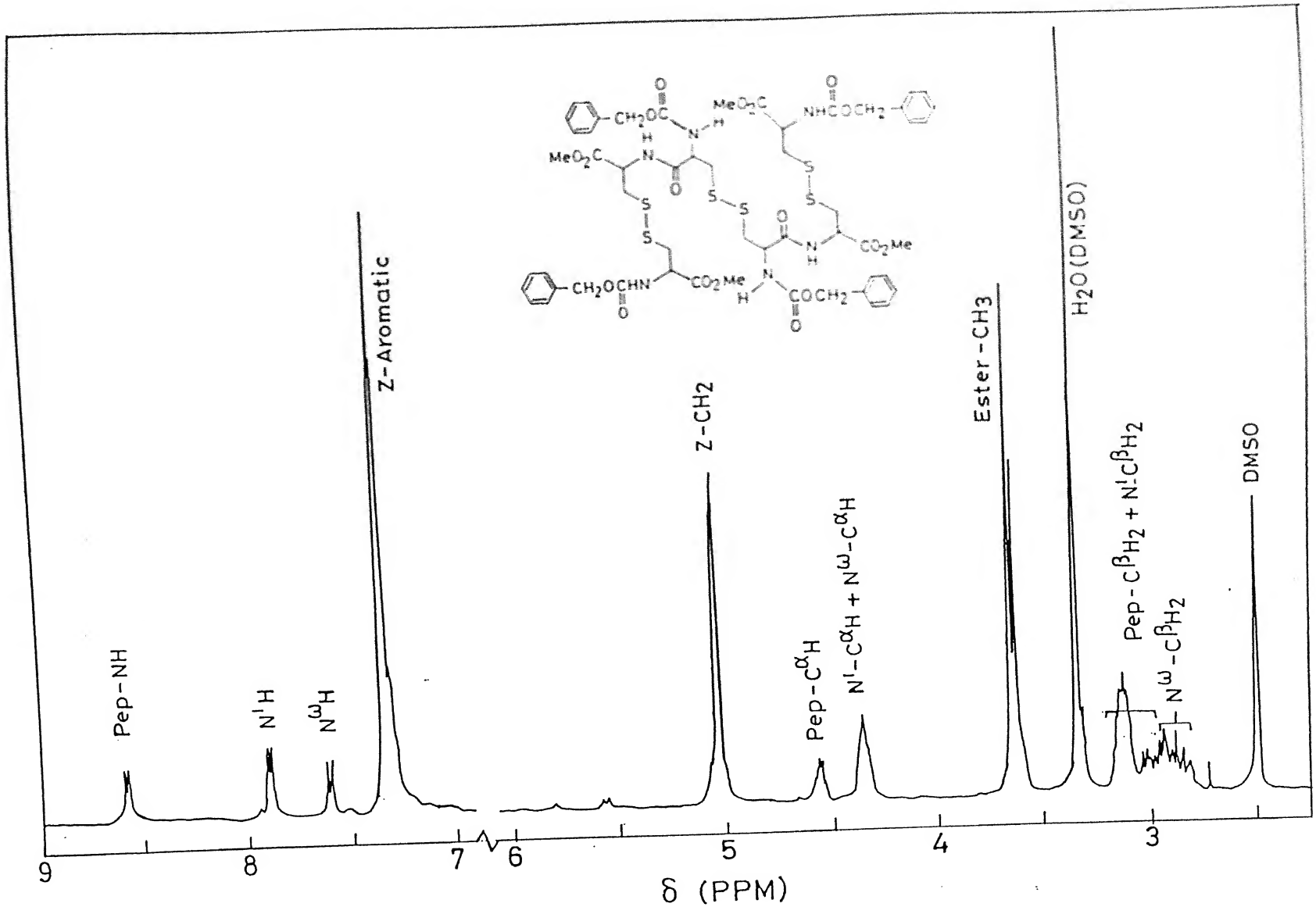
80 MHz ^{1}H-NMR spectrum of (97) in $CDCl_3$



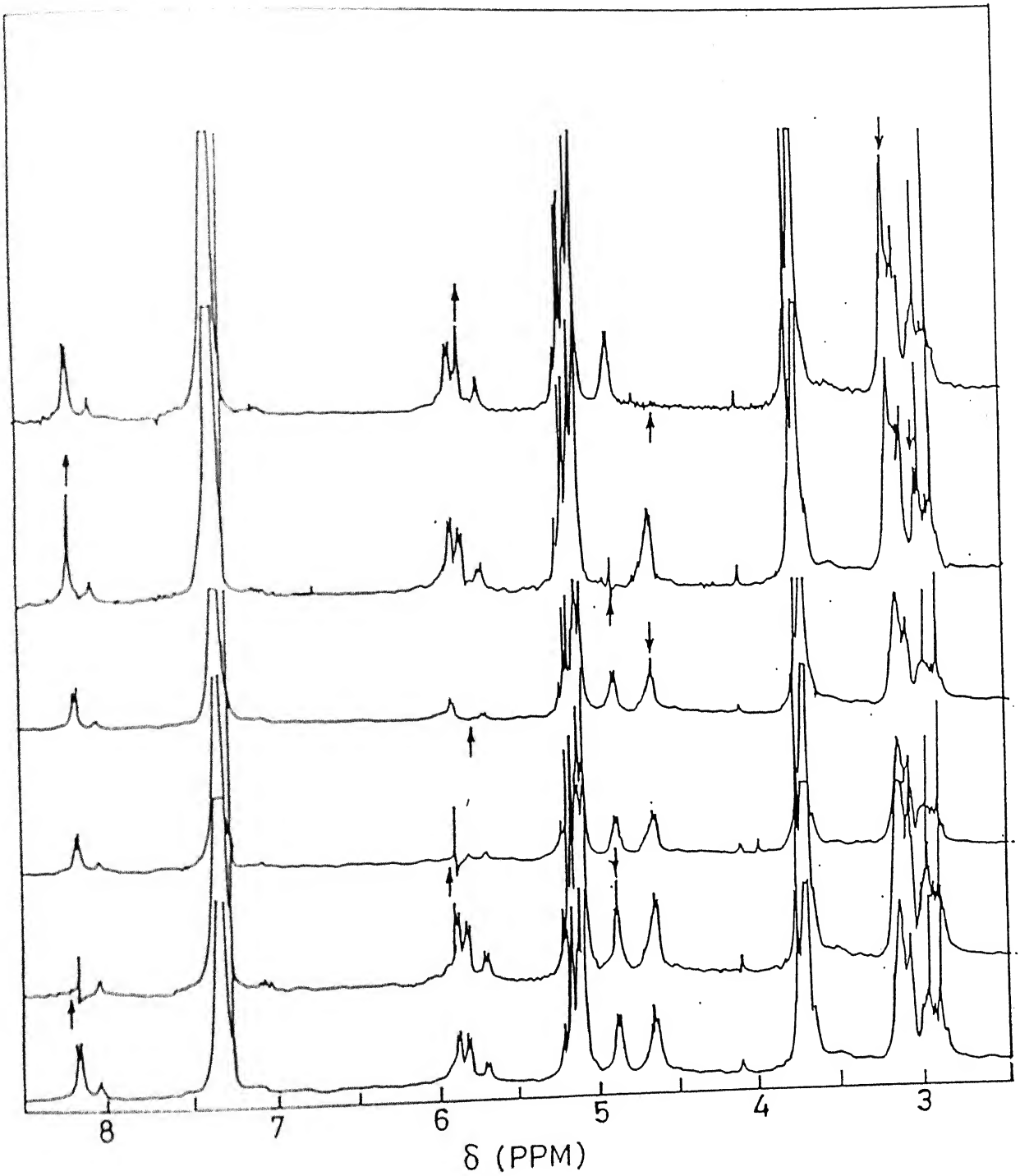
80 MHz ^{1}H Exchange NMR spectrum of (97) in $CDCl_3$ and D_2O



400 MHz ^{1}H-NMR spectrum of (97) in $CDCl_3$

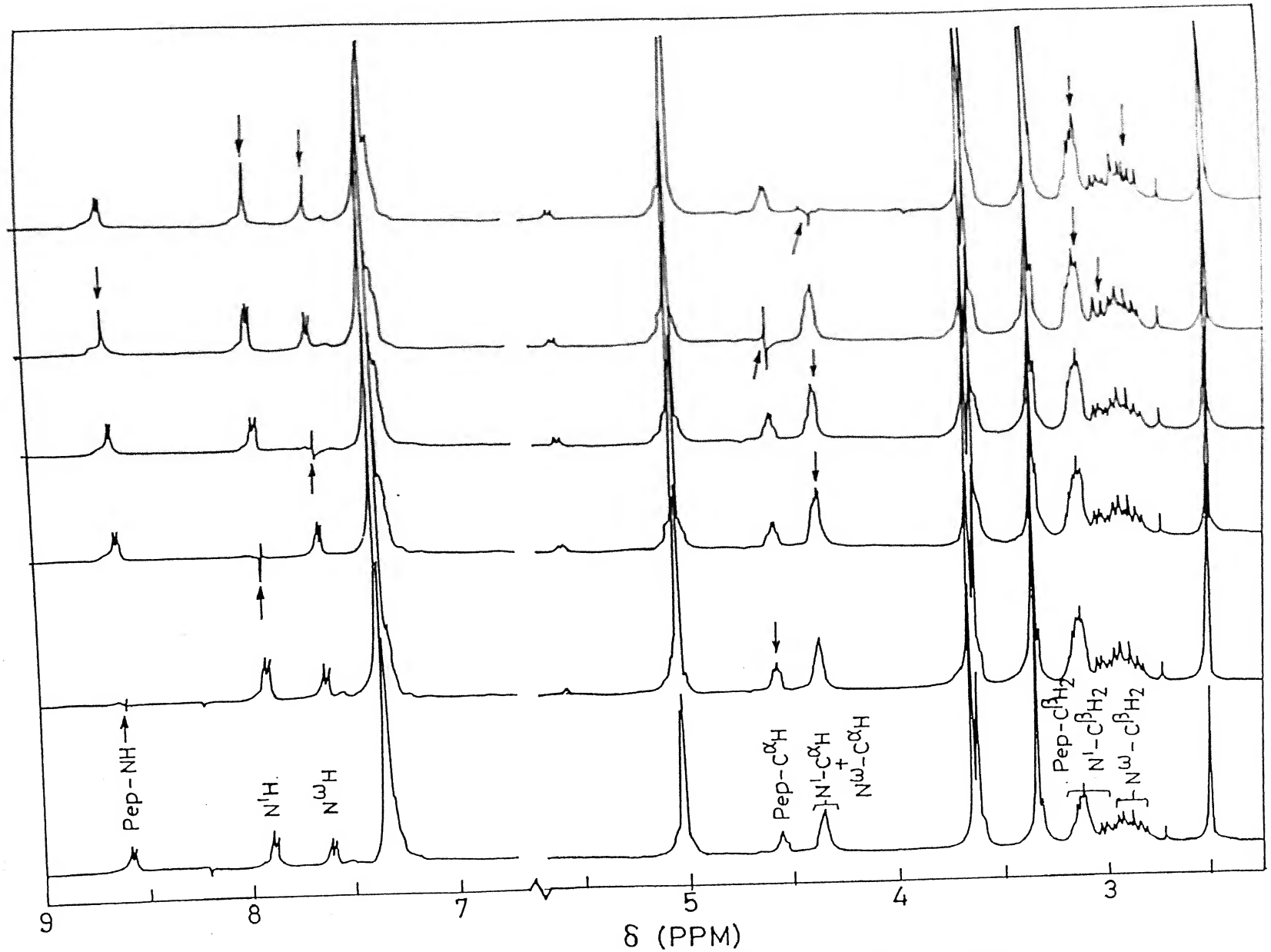


400 MHz ^{1}H-NMR spectrum of (97) in DMSO-d_6

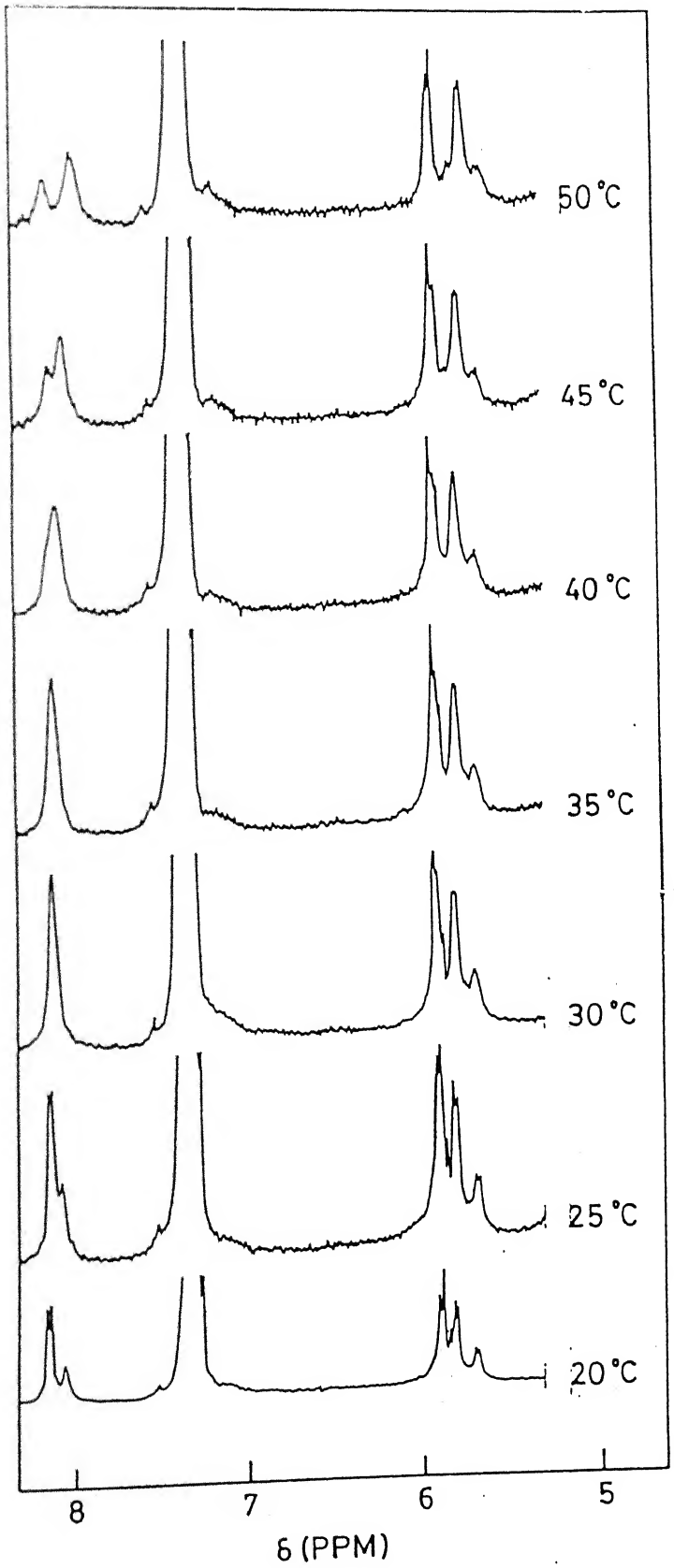


Spin Decoupling Experiments (400 MHz) on (97) in $CDCl_3$

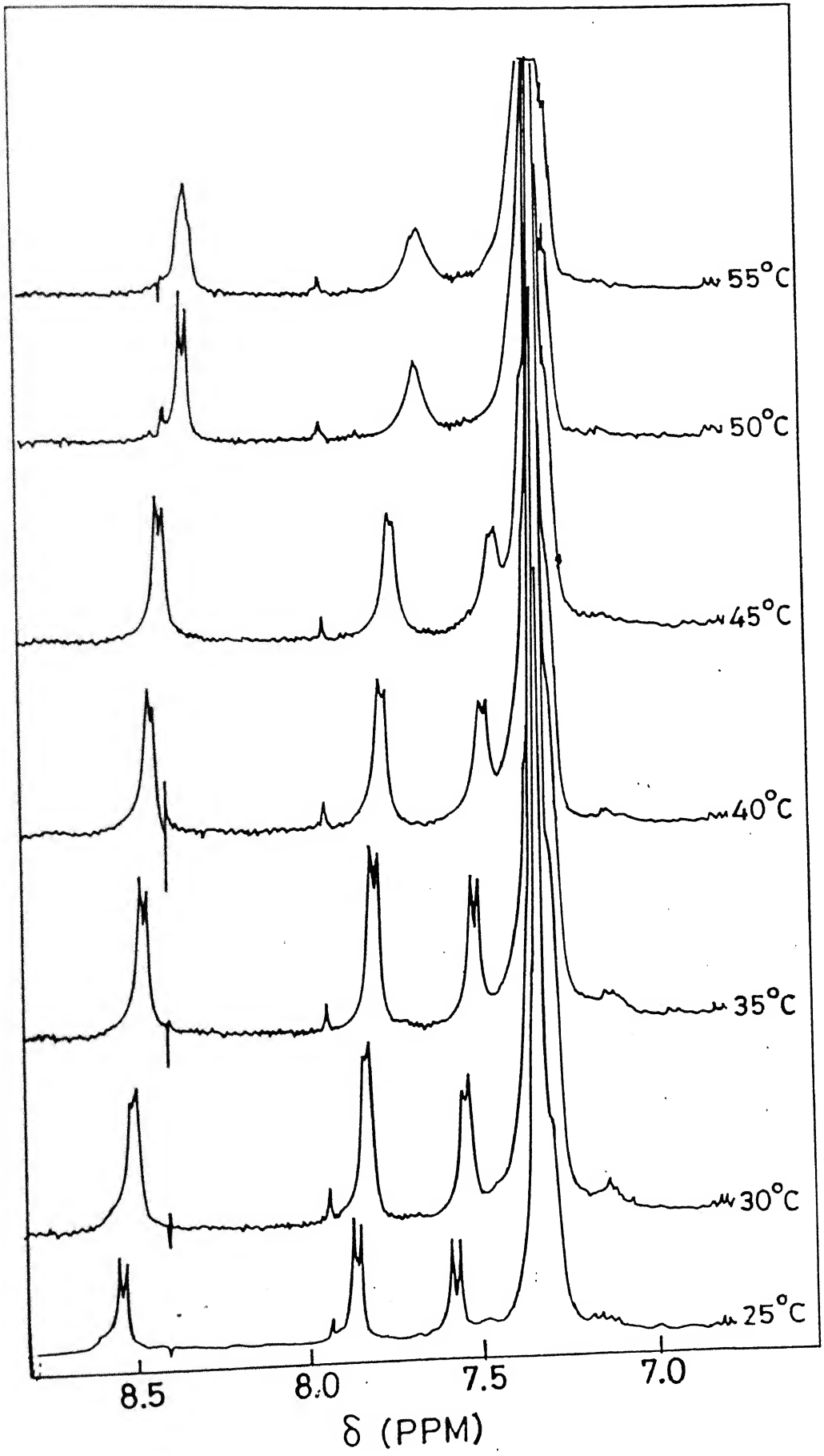
Upward arrows indicate the irradiated protons and downward arrows indicate the observed protons



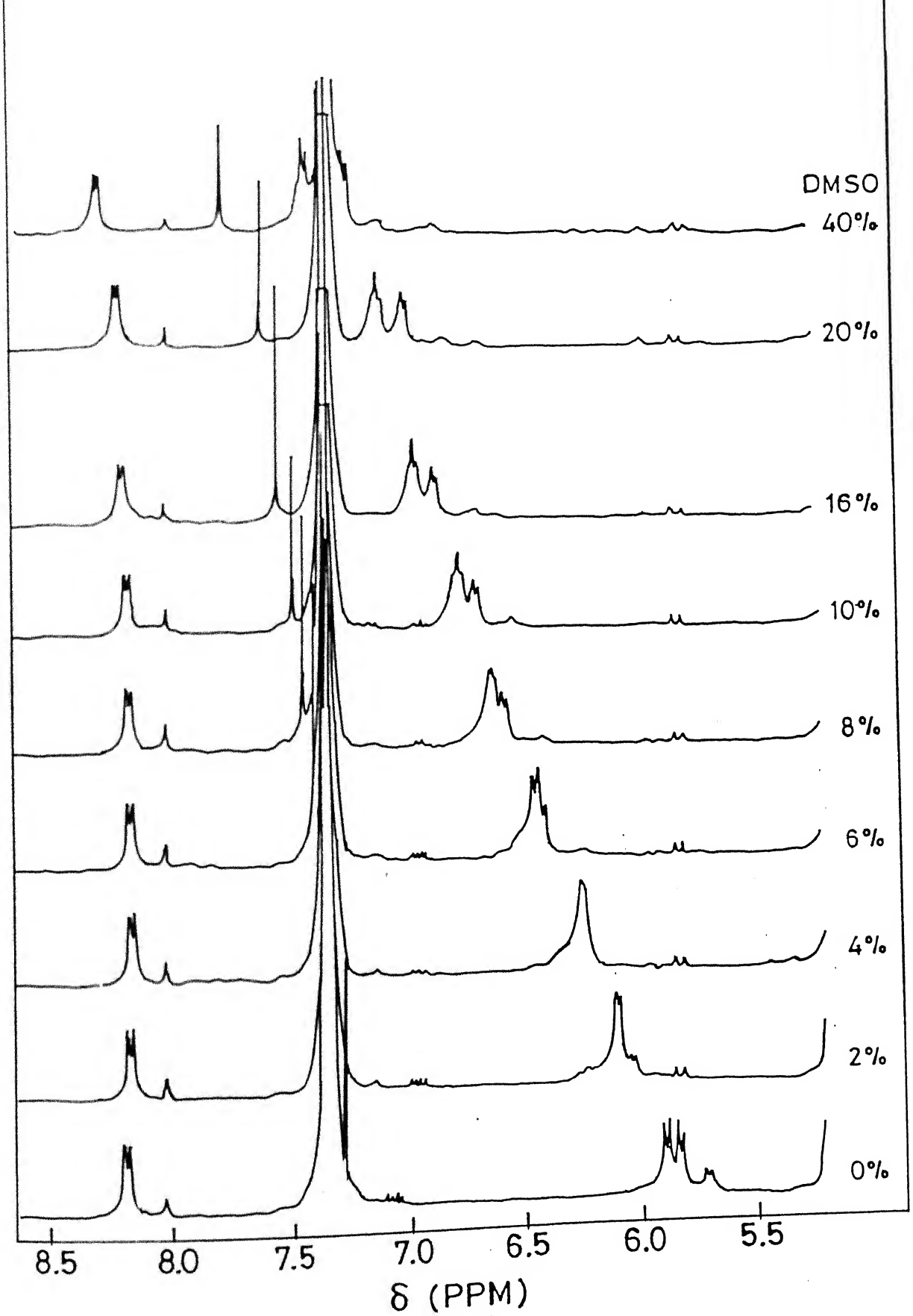
Spin Decoupling Experiments (400 MHz) on (97) in DMSO-d_6



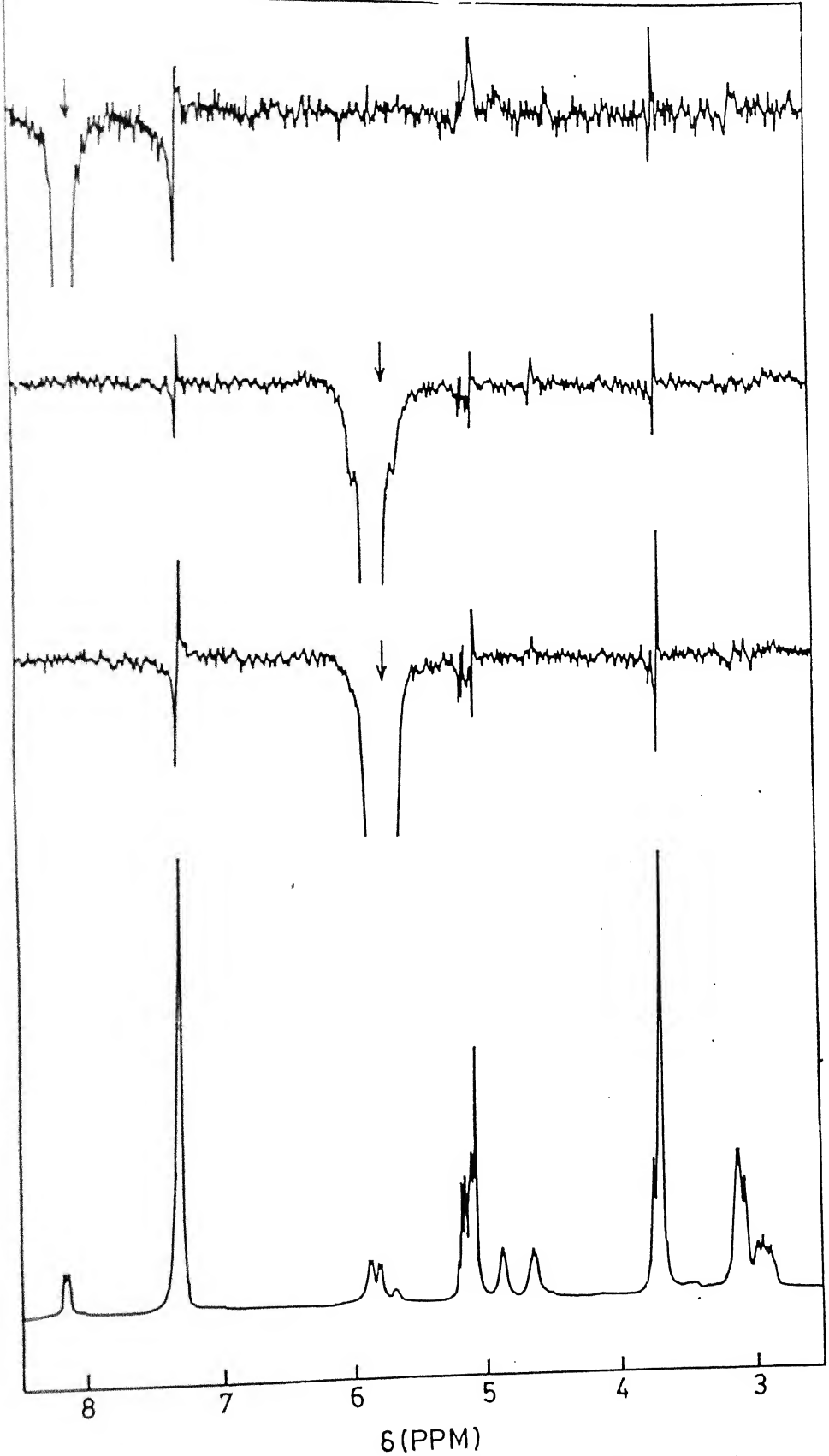
Variable Temperature ^{1}H-NMR (400 MHz) studies on (97) in $CDCl_3$



Variable Temperature ^{1}H-NMR (400 MHz) studies on (97) in DMSO-d_6

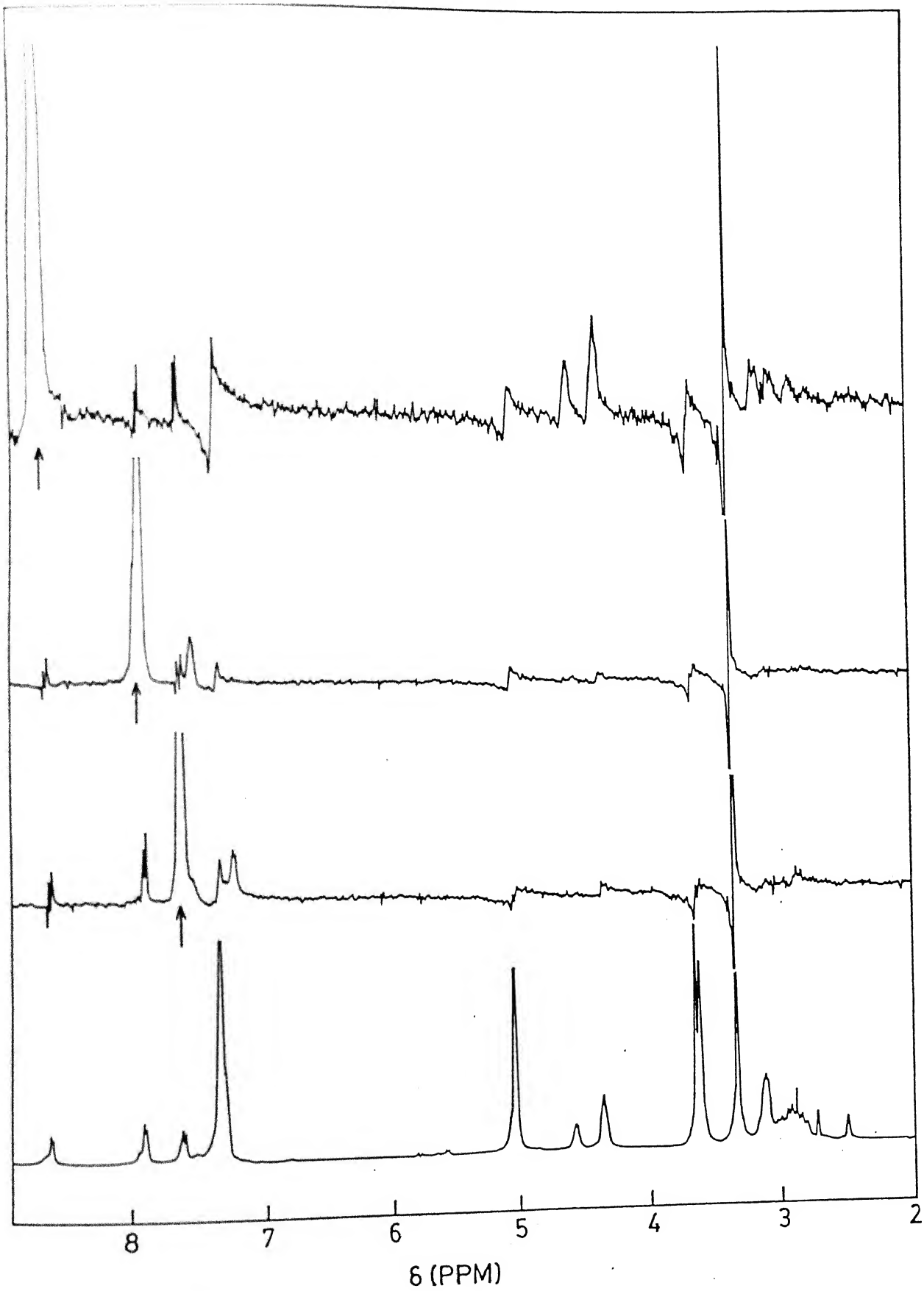


Solvent Dependence NMR studies (400 MHz) on (97) in $CDCl_3$ and DMSO-d_6



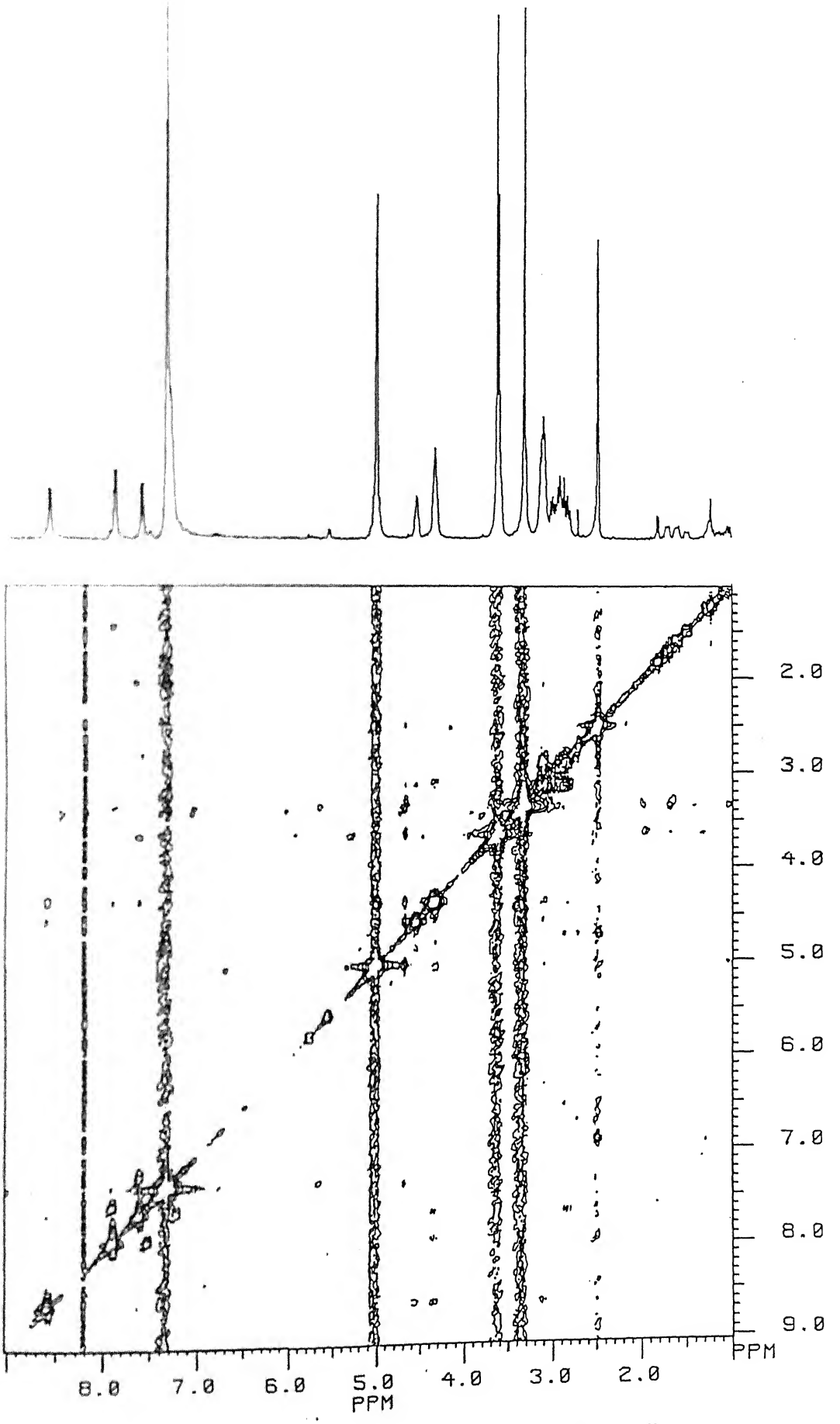
Difference NOE spectra (400 MHz) of (97) in $CDCl_3$

Arrows indicate the irradiated protons



Difference NOE spectra (400 MHz) of (97) in DMSO-d_6

Arrows indicate the irradiated protons



NOESY spectrum (400 MHz) of (97) in DMSO-d_6

E. EXPERIMENTAL

General: All amino acids used were of L-configuration. Proton NMR spectra were recorded on WM 400 Bruker instrument at 400 MHz, WP 80 Bruker instrument at 80 MHz and Hitachi R600 at 60 MHz. 2D proton NMR were recorded in WM 400 Bruker instrument. The chemical shifts are recorded in ppm with TMS at 0 as internal standard or as external reference. IR spectra were recorded on PE 580/1600 FT instruments, either as neat liquids or KBr pellets. Electronic spectra were recorded using PE-Lambda-2 UV-VIS spectrophotometer. Optical rotations were measured using an automatic JASCO digital polarimeter. FAB mass spectra were recoreded on a Jeol SX 102/DA-6000 mass spectrometer data system using argon (6KV, 10 mA) as the FAB gas. The accelerating voltage was 10 KV and the spectra were recorded at room temperature with m-nitrobenzyl alcohol as the matrix. EPR were recorded on a varian E-109 spectrometer operating at the X-band using DPPH as the external standard, at room temperature and liquid nitrogen temperature (77 K). Elemental analyses were carried out in automatic C, H, N analyser. Silica gel G (Merk) was used for tlc. Reactions were monitored wherever possible by tlc. The organic extracts were invariably dried over anhyd. $MgSO_4/Na_2SO_4$ and solvents evaporated *in vacuo*.

General Procedures

A. Preparation of 3-Oximinoacetyl-Tyrosine Compounds :

A stirred mixture of 3-acetyl-tyrosine containing compound (1 mmol) and hydroxylamine hydrochloride (2 mmol) in MeOH (15 mL) was treated with an aqueous solution of $NaHCO_3$ (2 mmol in 3 mL) at 25°C for 12 h. Solvents were evaporated, extracted with EtOAc (15 mL), washed with water (2 × 5 mL), dried ($MgSO_4/Na_2SO_4$), evaporated and recrystallized from ($MeOH:H_2O$ :: 3:1) to afford the oximino compounds.

B. Preparation of 3-Acetyl-Tyrosine - AEH Schiff Bases :

A solution of 3-acetyl-tyrosine containing compound (1 mmol) in MeOH (15 mL) was admixed with AEH (1 mmol) in MeOH (5 mL). The reaction mixture was left stirred at 25°C for 10 h, evaporated and crystallized form MeOH to give the desired product.

C. Preparation of 3-Acetyl-Tyrosine - Ethylenediamine Bis-Schiff Bases :

A solution of 3-acetyl-tyrosine containing compound (2 mmol) in MeOH (20 mL) was admixed with ethylenediamine (1 mmol) in MeOH (5 mL). The reaction mixture was held at 50°C for 2 h and left aside at 25°C for 10 h. The crystallized yellow solid was filtered and dried.

D. Preparation of Metal Templates from 3-Oximinoacetyl-Tyrosine Compounds :

A solution of the oxime (2 mmol) in MeOH (15 mL) was admixed with a solution of $M(OAc)_2$ (1 mmol) (M = Cu, Ni and Co) in $MeOH/H_2O$ (3 mL). The reaction mixture was left stirred at room temperature for 0.5 h, the precipitated metal complex filtered, thoroughly washed with water, MeOH and dried. In case of Cobalt, solvents evaporated and recrystallized from $MeOH\text{-}H_2O$.

E. Preparation of Metal Templates from 3-Acetyl-Tyrosine - AEH Schiff Bases :

A solution of AEH Schiff base compound (1 mmol) in MeOH (25 mL), was admixed with $M(OAc)_2$ (M = Cu, Ni and Co) (1 mmol) in MeOH (5 mL), held at 60°C for 0.5 h, evaporated and crystallized from MeOH.

F. Preparation of Metal Templates from 3-Acetyl-Tyrosine - Ethylenediamine (EDA) Schiff Bases :

A solution of EDA bis-Schiff base compound (1 mmol) in MeOH or MeCN (70 mL) was admixed with $M(OAc)_2$ (1 mmol) (M = Cu, Ni and Co) in MeOH (5 mL), held at 60°C for 0.5 h, evaporated and crystallized from MeOH.

G. Synthesis of peptides :

1-Hydroxybenzotriazole (HOBt, 1 mmol) and dicyclohexylcarbodiimide (DCC, 1 mmol) were added sequentially at 0°C to a stirred solution of N-protected amino acid (1 mmol) in dry CH_2Cl_2 (20 mL) and /or in dry DMF (5 mL). After a period of 0.25 h, the reaction mixture was admixed with the amino acid methyl ester, prepared at 0°C from the corresponding ester hydrochloride (1 mmol) and triethylamine (1.2 mmol) in dry CH_2Cl_2 or DMF. The combined mixture was left stirred at room temperature for 48 h, the precipitated DC urea filtered, residue washed with CH_2Cl_2 (2 × 10 mL) and the combined filtrates washed with CH_2Cl_2, cold 2N H_2SO_4 (20 mL), water (20 mL) and saturated sodium bicarbonate solution (20 mL). The organic extract was dried ($MgSO_4$) and evaporated *in vacuo*. The residue was directly crystallized from EtOAc-hexane mixture or purified on a short column of silica gel using PhH - EtOAc as eluents.

I. 3-Acetyl Tyrosine Hydrochloride [Tyr(3-Ac)HCl] (1) :

A stirred suspension of L-tyrosine (10 g, 0.055 mol) in nitrobenzene (240 mL) was admixed with $AlCl_3$ (28.8 g, 0.216 mol) and freshly distilled acetyl chloride (5.12 g, 0.065 mol). The reaction mixture was held at 100°C for 6 h, allowed to attain room temperature and poured onto a mixture of con. HCl (55 mL) and ice (340 g). The aqueous solution was concentrated to 200 mL and kept in the refrigerator. The separated solid was recrystallized from 5N HCl to give 11.3 g of (1).

yield	:	79%.
mp	:	220-223°C (dec.) (lit.[9] mp 220-224°C)
ir(KBr)ν_{max} cm^{-1}	:	3000 (br), 1742, 1640, 1582, 1518, 1499
nmr(D_2O) δ	:	2.3 (s, 3H, CH_3), 3.0 (d, 2H, $C^{\beta}H_2$), 4.12 (t, 1H, $C^{\alpha}H$), 6.71 (d, 1H, Tyr C-5 H), 7.26 (d, 1H, Tyr C-6 H), 7.54 (s, 1H, Tyr C-2 H)
ms (m/z)	:	224 $(MH)^+$-HCl
$[\alpha]_D^{25}$	:	-2.33° (c, 1.0, MeOH)

II. 3-Acetyl Tyrosine Methyl Ester Hydrochloride [Tyr(3-Ac)OMe.HCl] (2):

Dry HCl gas was passed through a stirred suspension of Tyr(3-Ac)HCl (1) (9.5 g, 43 mmol) in 100 mL of dry methanol for 0.5 h. Stirring was continued at room temperature for 1 h, the solvents evaporated *in vacuo* to give 9.9 g of a yellow solid, which was crystallized from MeOH-CH_2Cl_2.

yield	:	99%.
mp	:	186-187°C (dec.) (lit.[9] mp 180-183°C)
ir(KBr)ν_{max} cm^{-1}	:	3428, 2923, 1751, 1637, 1490
nmr(D_2O) δ	:	2.5 (s, 3H, CH_3), 3.12 (d, 2H, $C^{\beta}H_2$), 3.72 (s, 3H, $COOCH_3$), 6.87 (d, 1H, Tyr C-5 H), 7.3 (d, 1H, Tyr C-6 H), 7.67 (s, 1H, Tyr C-2 H)
ms (m/z)	:	238 $(MH)^+$-HCl
$[\alpha]_D^{25}$	:	-3.43° (c, 0.3, MeOH)

III. N-Benzoyl 3-Acetyl Tyrosine Methyl Ester [BzTyr(3-Ac)OMe] (3) :

Tyr(3-Ac)OMe.HCl (2) (0.8 g, 2.93 mmol) in a two-phase mixture of water (15 mL) and ether (12 mL) at 25°C was treated with Na_2CO_3 (0.938 g, 8.79 mmol) and benzoyl chloride (0.34 mL, 2.93 mmol), and the resulting mixture was stirred for 0.5 h. EtOAc (20 mL) was added to this mixture and the organic layers separated, washed with saturated aq. NaCl solution, dried over Na_2SO_4 and evaporated to give 0.863 g of (3) which was recrystallized from EtOAc-hexane.

yield	:	86%.
mp	:	131-133°C
ir(KBr)ν_{max} cm^{-1}	:	3443, 3068, 1750, 1637, 1580, 1517
nmr($CDCl_3$) δ	:	2.51 (s, 3H, CH_3), 3.31 (d, 2H, $C^{\beta}H_2$), 3.84 (s, 3H, $COOCH_3$), 5.12 (q, 1H, $C^{\alpha}H$), 6.65 (d, 1H, NH), 6.87-7.93 (m, 8H, aromatic), 12.19 (s, 1H, OH)
ms (m/z)	:	342 $(MH)^+$
anal	:	Calcd. for $C_{19}H_{19}NO_5$ (M.W. 341)
		C, 66.86; H, 5.57; N, 4.10 %
		Found : C, 66.07; H, 5.89; N, 4.25 %

IV. N-Benzoyl O-Acetyl Tyrosine Methyl Ester [BzTyr(O-Ac)OMe] (3a) :

a. TyrOMe.HCl :

To stirred and ice-cooled MeOH (35 mL) was added $SOCl_2$ (4 mL, 58 mmol) followed by tyrosine (9 g, 50 mmol). The reaction mixture was allowed to attain room temperature, stirred for additional 2 h and refluxed for 2 h. Solvents were evaporated and the residue crystallized from MeOH-Et_2O to give TyrOMe.HCl.

b. N-Benzoyl Tyrosine Methyl Ester :

TyrOMe.HCl (1.0 g, 4.33 mmol) in a two phase mixture of water (18 mL) and ether (15 mL) at 25°C was admixed with Na_2CO_3 (1.38 g, 13 mmol) and benzoylchloride (0.5 mL, 4.33 mmol) and the resulting mixture was stirred for 0.5 h. The ether layer was separated, washed with saturated aq. NaCl solution, dried ($MgSO_4$) and evaporated to give BzTyrOMe.

c. N-Benzoyl-O-Acetyl Tyrosine Methyl Ester :

To a stirred suspension of BzTyrOMe (0.5 g, 1.67 mmol) in acetic anhydride (4 mL) was added pyridine (0.3 mL, 3.69 m mol). After stirring for 3 h at 25°C, water (15 mL) was added and extracted with EtOAc (3 x 15 mL). The EtOAc extract was washed with 2N HCl (2 x 5 mL), saturated aq. Na_2CO_3 and evaporated to give 0.472 g of (3a).

yield	:	83%.
mp	:	118°C
ir(KBr)ν_{max}cm^{-1}	:	3317, 1740, 1639, 1512
nmr($CDCl_3$) δ	:	2.34 (s, 3H, $OCOCH_3$), 3.31 (d, 2H, $C^{\beta}H_2$), 3.81 (s, 3H, $COOCH_3$), 5.12 (d, d, 1H, $C^{\alpha}H$), 6.75 (d, 1H, NH), 6.93-7.90 (m, 9H, aromatic)

V. N-Benzyloxycarbonyl 3-Acetyl Tyrosine Methyl Ester [ZTyr(3-Ac)OMe] (4) :

Tyr(3-Ac)OMe.HCl (2) (5.46 g, 20 mmol) in a two phase mixture of 100 mL of water and 80 mL of ether at 25°C was treated with Na_2CO_3 (6.4 g, 60.0 mmol) and benzyl chloroformate (2.9 mL, 20 mmol) and the resulting mixture was stirred for 1 h. The ether layer was separated, washed with saturated aq. NaCl, dried ($MgSO_4$) and evaporated to afford 6.68 g of (4).

yield	:	98%
mp	:	93-95° (lit.[9] mp 94-96°C)
ir(KBr)ν_{max} cm^{-1}	:	3308, 1745, 1685, 1642, 1541
nmr($CDCl_3$) δ	:	2.53 (s, 3H, CH_3), 3.13 (m, 2H, $C^{\beta}H_2$), 3.78 (s, 3H, $COOCH_3$), 4.63 (d d, 1H, $C^{\alpha}H$), 5.13 (s, 2H, Z-CH_2), 5.31 (d, 1H, NH), 6.81-7.63 (m, 8H, aromatic), 12.16 (s, 1H, OH)
ms (m/z)	:	372$(MH)^+$

VI. N,O,3-Triacetyl Tyrosine Methyl Ester [AcTyr(O,3-diAc)OMe] (5) :

To compound (2) (1.2 g, 4.4 mmol) in Ac_2O (4 mL) was added pyridine (1.0 mL, 12.3 m mol) and stirred at 25°C for 8 h. The clear solution was then admixed with 10% HCl (10 mL) and extracted with EtOAc (3 x 15 mL). The EtOAc extract was repeatedly washed with saturated aq. $NaHCO_3$ solution and water, dried ($MgSO_4$) and evaporated to give 1.13 g of (5).

yield	:	80%
mp	:	96°C
ir(KBr)ν_{max} cm^{-1}	:	3306, 2954, 1751, 1685, 1647, 1580, 1548
nmr($CDCl_3$) δ	:	2.0 (s, 3H, $NCOCH_3$), 2.34 (s, 3H, $OCOCH_3$), 2.53 (s, 3H, $COCH_3$), 3.19 (d, 2H, $C^{\beta}H_2$), 3.78 (s, 3H, $COOCH_3$), 4.91 (q, 1H, $C^{\alpha}H$), 6.19 (d, 1H, NH), 6.97-7.63 (m, 3H, aromatic)
ms (m/z)	:	322 $(MH)^+$-HCl
anal	:	Calcd. for $C_{16}H_{19}NO_6Cl$ (M.W. 321)
		C, 59.81; H, 5.91; N, 4.36 %
		Found : C, 60.27; H, 6.14; N, 4.87 %

VII. N,3-Diacetyl Tyrosine Methyl Ester [AcTyr(3-Ac)OMe] (6) :

A solution of (5) (0.321 g, 1 mmol) in methanol (10 mL) was admixed with aqueous solution of $NaHCO_3$ (0.084 g, 1 mmol, 5 mL) and stirred at room temperature for 4 h. MeOH was evaporated *under vacuo* and the aqueous solution acidified with 10 % HCl to pH 3, extracted with EtOAc (3 × 15 mL) and the EtOAc extract washed with saturated aq. $NaHCO_3$ solution (2 × 10 mL), dried ($MgSO_4$) and evaporated to give 0.267 g of (6) as a white solid.

yield	:	83%
mp	:	136°C
ir(KBr)ν_{max} cm^{-1}	:	3299, 3070, 1733, 1650, 1595, 1544
nmr($CDCl_3$) δ	:	2.03 (s, 3H, $NCOCH_3$), 2.63 (s, 3H, $COCH_3$), 3.16 (d, 2H, $C^{\beta}H_2$), 3.81 (s, 3H, $COOCH_3$), 4.91 (d, 1H, $C^{\alpha}H$), 6.0 (d, 1H, NH), 6.97 (d, 1H, Tyr C-5 H), 7.25 (d, 1H, Tyr C-6 H), 7.53 (s, 1H, Tyr C-2 H), 12.16 (s, 1H, OH)
ms (m/z)	:	280 $(MH)^+$
anal	:	Calcd. for $C_{14}H_{17}NO_5$ (M.W. 279)
		C, 60.21; H, 6.09; N, 5.01 %
		Found : C, 59.69; H, 6.22; N, 5.14 %

VIII. N-Benzoyl-3-Oximinoacetyl Tyrosine Methyl Ester [BzTyr(3-Oximinoacetyl)OMe] (7) :

BzTyr(3-Ac)OMe (3) (0.17 g, 0.5 mmol) by General Procedure-A gave 0.14 g of (7).

yield	:	79%
mp	:	172°C
ir(KBr)ν_{max} cm^{-1}	:	3318, 1738, 1706, 1634, 1580, 1526, 1492
nmr($CDCl_3$) δ	:	2.26 (s, 3H, oximino CH_3), 3.2 (d, 2H, $C^{\beta}H_2$), 3.8 (s, 3H, $COOCH_3$), 5.0 (m, 1H, $C^{\alpha}H$), 6.73 -7.93 (m, 9H, NH + aromatic), 11.0 (s, 1H, N-OH), 11.8 (s, 1H, phenolic OH)
ms (m/z)	:	357 $(MH)^+$
anal	:	Calcd. for $C_{19}H_{20}N_2O_5$ (M.W. 356)
		C, 64.04; H, 5.61; N, 7.86 %
		Found : 64.16; H, 5.83; N, 7.89 %

IX. N-Benzyloxycarbonyl-3-Oximinoacetyl Tyrosine Methyl Ester [ZTyr(3-Oximinoacetyl)OMe] (8) :

ZTyr(3-Ac)OMe (4) (0.371 g, 1 mmol) by General Procedure-A gave 0.352 g of (8).

yield	:	91%
mp	:	101-102°C
ir(KBr)ν_{max} cm^{-1}	:	3379, 2922, 2851, 1718, 1686, 1637, 1541
nmr($CDCl_3$) δ	:	2.30 (s, 3H, oximino CH_3), 3.1 (d, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.65 (q, 1H, $C^{\alpha}H$), 5.15 (s, 2H, Z CH_2), 5.5 (d, 1H, NH), 6.9-7.5 (m, 8H, aromatic), 11.6 (s, 1H, N-OH), 12.1 (s, 1H, phenolic OH)
ms (m/z)	:	387 $(MH)^+$

X. N-Acetyl-3-Oximinoacetyl Tyrosine Methyl Ester [AcTyr(3-Oximinoacetyl)-OMe] (9) :

AcTyr(3-Ac)OMe (6) (0.139 g, 0.5 mmol) by General Procedure-A gave 0.123 g of (9).

yield	:	84%
mp	:	135°C
ir(KBr)ν_{max} cm^{-1}	:	3446, 3313, 2931, 1740, 1648, 1535, 1494

nmr($CDCl_3$) δ	:	1.97 (s, 3H, $NCOCH_3$), 2.31 (s, 3H, oximino CH_3), 3.06 (d, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.75 (q, 1H, $C^{\alpha}H$), 6.69-7.19 (m, 4H, NH + aromatic), 10.72 (s, 1H, N-OH), 11.59 (s, 1H, phenolic OH)
ms (m/z)	:	295 $(MH)^+$
anal	:	Calcd. for $C_{14}H_{18}N_2O_5$ (M.W.294) C, 57.14; H, 6.12; N, 9.52 % Found : C, 57.28; H, 6.36; N, 9.83 %

XI. Acetylacetone - Ethylenediamine Mono-Schiff Base [AEH][44] (11) :

Under vigorous stirring, a solution of acetylacetone (0.1 mol) in $CHCl_3$ (50 mL) was added, in drops, to a solution of ethylenediamine (0.1 mol) in $CHCl_3$ (100 mL). The reaction mixture was left stirred for 10 h, separated water removed, solvents evaporated and the crude product used as such without delay.

yield	:	84%
mp	:	liquid
ir(neat)ν_{max} cm^{-1}	:	3289, 1734, 1609, 1560, 1437, 1325
nmr($CDCl_3$) δ	:	1.66 (m, 2H, NH_2), 2.02 (s,s, 6H, CH_3 x 2), 2.9 (m, 2H, CH_2), 3.38 (m, 2H, =N-CH_2) 5.03 (s, 1H, CH), 10.97 (s, 1H, OH)

XII. Preparation of N-Benzoyl 3-Acetyl Tyrosine Methyl Ester - AEH Schiff Base (12) :

BzTyr(3-Ac)OMe (3) (0.682 g, 2 mmol) by General Procedure-B gave 0.558 g of (12).

yield	:	60%
mp	:	190-191°C
ir(KBr)ν_{max} cm^{-1}	:	3273, 3058, 2948, 1747, 1641, 1605, 1582, 1558
nmr($CDCl_3$) δ	:	1.98 (s, s, 6H, AEH CH_3 x 2), 2.22 (s, 3H, CH_3), 3.22 (d, 2H, $C^{\beta}H_2$), 3.56-3.97 (m, 7H, -CH_2CH_2- + $COOCH_3$), 5.0 (m, 2H, $C^{\alpha}H$ + enolic-CH), 6.66 (d, 1H, NH), 6.75-7.9 (m, 8H, aromatic), 10.97 (s, 1H, enolic OH), 15.37 (s, 1H, phenolic OH)
ms (m/z)	:	466 $(MH)^+$

uv-vis ($CHCl_3$)λ_{max} nm	:	239, 312
anal	:	Calcd. for $C_{26}H_{31}N_3O_5$ (M.W. 465) C, 67.09; H, 6.66; N, 9.03 % Found : C, 66.43; H, 6.39; N, 8.96 %

XIII. Preparation of Bis-N-Benzoyl 3-Acetyl Tyrosine Methyl Ester - Ethylenediamine Schiff Base (13) :

BzTyr(3-Ac)OMe (3) (0.341 g, 1 mmol) by General Procedure-C afforded 0.245 g of (13).

yield	:	70%
mp	:	243-245°C
ir(KBr)ν_{max} cm^{-1}	:	3310, 2953, 1747, 1645, 1632, 1578
nmr($CDCl_3$-TFA) δ	:	2.84 (s, 6H, CH_3 x 2), 3.31 (d, 4H, $C^{\beta}H_2$ x 2), 3.91 (s, 6H, $COOCH_3$ x 2), 4.47 (s, 4H, -CH_2CH_2-), 5.16 (q, 2H, $C^{\alpha}H$ x 2), 7.0-7.88 (m, 16H, aromatic)
ms (m/z)	:	707 $(MH)^+$
anal	:	Calcd. for $C_{40}H_{42}N_4O_8$ (M.W. 706) C, 67.98; H, 5.94; N, 7.93 % Found : C, 67.27; H, 5.81; N, 7.8 %

XIV. Bis-N-Benzyloxycarbonyl 3-Acetyl Tyrosine Methyl Ester - Ethylenediamine Schiff Base (14) :

ZTyr(3-Ac)OMe (4) (0.371 g, 1 mmol) by General Procedure-C afforded 0.314 g of (14).

yield	:	82%
mp	:	204°C
ir(KBr)ν_{max} cm^{-1}	:	3412, 1746, 1686, 1619
nmr($CDCl_3$) δ	:	1.37 (s, 6H, CH_3 × 2), 3.15 (d, 4H, $C^{\beta}H_2$ × 2), 3.78 (s, 6H, $COOCH_3$ × 2), 4.0 (s, 4H, -CH_2CH_2-), 4.68 (q, 2H, $C^{\alpha}H$ × 2), 5.18 (s, 4H, Z CH_2 × 2), 5.3 (d, 2H, NH × 2), 6.93-7.53 (m, 16H, aromatic)

XV. N-^{t}Butyloxycarbonyl Alanine [BocAla] (15) :

To an ice-cooled and well stirred solution of alanine (3.56 g, 40 mmol) in aq. NaOH (1.85 g, 46.25 mmol in 24 mL H_2O) was added dioxane (25 mL) followed by Boc-azide (60 mmol) and left stirred for 24 h at room temperature. The reaction mixture was diluted with ice-water (~50 mL) and extracted with ether (2 x 25 mL). The aqueous phase was acidified with solid citric acid to pH 3, saturated with solid NaCl, extracted with EtOAc (3 x 50 mL), dried ($MgSO_4$), solvents evaporated *in vacuo* and the residue crystallized from EtOAc/hexane to give BocAla (15).

yield	:	78%
mp	:	80°C (lit.[45] mp 82-84°C)
ir(KBr)ν_{max} cm^{-1}	:	3383, 2989, 1692, 1518, 1456

XVI. N-^{t}Butyloxycarbonyl Alanyl 3-Acetyl Tyrosine Methyl Ester [BocAla-Tyr(3-Ac)OMe] (16) :

DCC coupling of BocAla (15) (1.6 g, 8.5 mmol) with Tyr(3-Ac)OMe.HCl (2) (2.45 g, 9 mmol) afforded 2.31 g of BocAlaTyr(3-Ac)OMe (16), [General Procedure-G].

yield	:	67%
mp	:	106°C
ir(KBr)ν_{max} cm^{-1}	:	3340, 2982, 1750, 1685, 1651, 1525
nmr($CDCl_3$) δ	:	1.28 (d, 3H, Ala CH_3), 1.37 (s, 9H, Boc CH_3), 2.63 (s, 3H, $COCH_3$), 3.1 (d, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.06 (m, 1H, Ala $C^{\alpha}H$), 4.84 (m, 2H, Tyr $C^{\alpha}H$ + Ala NH), 6.63 (d, 1H, Tyr NH), 6.9 (d, 1H, Tyr C-5 H), 7.22 (d, 1H, Tyr C-6 H), 7.53 (s, 1H, Tyr C-2 H), 11.16 (s, 1H, OH)
ms (m/z)	:	409 $(MH)^+$
$[\alpha]_D^{25}$	:	-5.96° (c, 1.0, MeOH)
anal	:	Calcd. for $C_{20}H_{28}N_2O_7$ (M.W. 408) C, 58.8; H, 6.86; N, 6.86% Found : C, 58.89; H, 7.02; N, 7.13%

XVII. N-^{t}Butyloxycarbonyl Alanyl 3-Acetyl Tyrosine [BocAlaTyr(3-Ac)] (17):

A solution of (16) (1.0 g, 2.45 mmol) in MeOH (15 mL) was cooled to 0°C, treated with aq. NaOH (2N, 7 mL) and stirred at room temperature for 4 h. The reaction mixture was concentrated to ~7 mL (without heating) *in vacuo*, cooled in ice and acidified (pH 3) with 10% HCl acid, extracted with EtOAc (3 x 25 mL), dried ($MgSO_4$) and evaporated *in vacuo* to give 0.945 g of the acid.

yield	:	98%
mp	:	hygroscopic
ir(KBr)ν_{max} cm^{-1}	:	3349, 2979, 2933, 1716, 1644, 1521, 1488
nmr($CDCl_3$-DMSO-d_6) δ	:	1.35 (d, 3H, Ala CH_3), 1.5 (s, 9H, Boc CH_3), 2.65 (s, 3H, $COCH_3$), 3.1 (d, 2H, $C^{\beta}H_2$), 4.2 (q, 1H, Ala $C^{\alpha}H$), 4.8 (q, 1H, Tyr $C^{\alpha}H$), 5.25 (d, 1H, Ala NH), 6.8 (d, 1H, Tyr NH), 7.0 (d, 1H, Tyr C-5 H), 7.3 (d, 1H, Tyr C-6 H), 7.55 (s, 1H, Tyr C-2 H), 11.6 (s, 1H, phenolic OH)
ms (m/z)	:	395 $(MH)^+$

XVIII. Serine Methyl Ester Hydrochloride [SerOMe.HCl] (18) :

To a stirred suspension of serine (100 mmol) in dry MeOH (75 mL), dry HCl was passed first at room temperature until a clear solution was obtained then the passage of HCl continued at 0°C until saturation. Solvents were evaporated *in vacuo* and the residue was redissolved in dry MeOH and again subjected to passage of dry HCl for 1 h, followed by evaporation of solvents and crystallization (MeOH-Et_2O) to afford (18) as white spongy solid.

yield	:	95%
mp	:	166 °C (lit.[46] mp 166°)C)
ir(KBr)ν_{max} cm^{-1}	:	3380, 1730

XIX. N-^{t}Butyloxycarbonyl Alanyl 3-Acetyl Tyrosyl Serine Methyl Ester [BocAlaTyr(3-Ac)SerOMe] (19) :

DCC coupling of BocAlaTyr(3-Ac) (17) (0.9 g, 2.3 mmol) with SerOMe.HCl (18)

(0.39 g, 2.5 mmol) by General Procedure-G afforded 0.36 g of BocAlaTyr(3-Ac)SerOMe (19).

yield	:	32%
mp	:	172°C
ir(KBr)ν_{max} cm^{-1}	:	3299, 2931, 1747, 1687, 1647, 1530
nmr($CDCl_3$) δ	:	1.31 (d, 3H, Ala CH_3), 1.47 (s, 9H, Boc CH_3), 2.65 (s, 3H, $COCH_3$), 3.16 (d, 2H, Tyr $C^{\beta}H_2$), 3.65 (s, 3H, $COOCH_3$), 3.81 (d, 2H, Ser $C^{\beta}H_2$), 4.13 (q, 1H, Ala $C^{\alpha}H$), 4.69 (m, 2H, Tyr $C^{\alpha}H$ + Ser $C^{\alpha}H$), 5.03 (d, 1H, Ala NH), 6.78-7.8 (m, 5H, aromatic + Tyr NH + Ser NH), 12.1 (s, 1H, phenolic OH)
ms (m/z)	:	496 $(MH)^+$
$[\alpha]_D^{25}$	:	-21.0° (c, 0.5, MeOH)
anal	:	Calcd. for $C_{23}H_{33}N_3O_9$ (M.W. 495) C, 55.75; H, 6.66; N, 8.48 % Found : C, 55.72; H, 6.54; N, 8.62 %

XX. N-Benzyloxycarbonyl Alanine [ZAla] (20) :

To an ice-cooled and well stirred solution of alanine (100 mmol) in 25 mL of 4N NaOH (100 mmol) was added 30 mL of 4N NaOH (120 mmol) and 18.7 g (100 mmol) of benzyloxycarbonyl chloride, alternately, in about 5 equal parts, over a period of 0.5 h. The reaction mixture was left stirred for 2 h at 0°C, extracted with ether (2 x 30 mL) to remove excess benzyloxycarbonyl chloride, the aqueous layer adjusted to pH 2 with 5N HCl, under cooling in an ice bath, extracted with EtOAc (3 x 30 mL), the organic layers dried ($MgSO_4$), solvents evaporated *in vacuo* and the residue crystallized from EtOAc/hexane to give (20).

yield	:	92%
mp	:	96-97°C (lit.[47] mp 97-99°C)
ir(KBr)ν_{max} cm^{-1}	:	3332, 3034, 1701, 1535
nmr($CDCl_3$) δ	:	1.44 (d, 3H, CH_3), 4.38 (m, 1H, $C^{\alpha}H$), 5.16 (s, 2H, Z CH_2), 5.47 (d, 1H, NH), 7.34 (s, 5H, aromatic), 9.69 (s, 1H, COOH)

XXI. N-Benzyloxycarbonyl Alanyl 3-Acetyl Tyrosine Methyl Ester [ZAlaTyr(3-Ac)OMe] (21) :

DCC coupling of ZAla (20) (2.007 g, 9 mmol) with Tyr(3-Ac)OMe.HCl (2) (2.5 g, 9.16 mmol) by General Procedure-G afforded 2.48 g of ZAlaTyr(3-Ac)OMe (21).

yield	:	62%
mp	:	163-164°C
ir(KBr)ν_{max} cm^{-1}	:	3309, 2942, 1741, 1691, 1643, 1526
nmr($CDCl_3$) δ	:	1.34 (d, 3H, Ala CH_3), 2.59 (s, 3H, $COCH_3$), 3.09 (d, 2H, $C^{\beta}H_2$), 3.78 (s, 3H, $COOCH_3$), 4.19 (m, 1H, Ala $C^{\alpha}H$), 4.84 (q, 1H, Tyr $C^{\alpha}H$), 5.06 (s, 2H, Z CH_2), 5.31 (d, 1H, Ala NH), 6.66 (d, 1H, Tyr NH), 6.88 (d, 1H, Tyr C-5 H), 7.22 (d, 1H, Tyr C-6 H), 7.38 (s, 5H, Z aromatic), 7.53 (s, 1H, Tyr C-2 H), 13.41 (s, 1H, OH)
ms (m/z)	:	443 $(MH)^+$
anal	:	Calcd. for $C_{23}H_{26}N_2O_7$ (M.W. 442) C, 62.44; H, 5.88; N, 6.33 % Found : C, 62.27; H, 6.13; N, 6.08 %

XXII. N-Benzyloxycarbonyl Alanyl 3-Acetyl Tyrosine [ZAlaTyr(3-Ac)] (22):

A solution of (21) (1.2 g, 2.7 mmol)in MeOH (15 mL) was cooled to 0°C, treated with aq. NaOH (2N, 7 mL) and stirred at room temperature for 4 h. The reaction mixture was concentrated to ~7 mL (without heating) *in vacuo*, cooled in ice and acidified (p^H 3) with 10% HCl acid, extracted with EtOAc (3 x 25 mL), dried ($MgSO_4$) and evaporated *in vacuo* to afford 0.977 g of the acid.

yield	:	85%
mp	:	184-187°C
ir(KBr)ν_{max} cm^{-1}	:	3282, 1732, 1681, 1640 , 1621, 1525

XXIII.Benzyloxycarbonyl Alanyl 3-Acetyl Tyrosyl Serine Methyl Ester [ZAlaTyr(3-Ac)SerOMe] (23) :

DCC coupling of ZAlaTyr(3-Ac) (22) (0.9 g, 2.1 mmol) with SerOMe.HCl (18) (0.341

g, 2.2 mmol) by General Procedure-G afforded 0.604 g of ZAlaTyr(3-Ac)SerOMe (23).

yield	:	54%
mp	:	174-176°C
ir(KBr)ν_{max} cm^{-1}	:	3319, 2927, 1743, 1687, 1641, 1538
nmr($CDCl_3$) δ	:	1.34 (d, 3H, Ala CH_3), 2.69 (s, 3H, $COCH_3$), 3.06 (d, 2H, Tyr $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 3.84 (d, 2H, Ser $C^{\beta}H_2$), 4.13 (m, 1H, Ala $C^{\alpha}H$), 4.28-4.94 (m, 2H, Tyr $C^{\alpha}H$ + Ser $C^{\alpha}H$), 5.06 (s, 2H, Z CH_2), 6.53 (d, 1H, Ala NH), 6.88 (d, 1H, Tyr C-5 H), 7.09-7.94 (m, 9H, Tyr C-6 H + Tyr C-2 H + Z aromatic + Tyr NH + Ser NH), 12.16 (s, 1H, phenolic OH)
ms (m/z)	:	530 $(MH)^+$
anal	:	Calcd. for $C_{26}H_{31}N_3O_9$ (M.W. 529) C, 58.97; H, 5.86; N, 7.93 % Found : C, 58.46; H, 6.13; N, 8.17 %

XXIV. N-Benzyloxycarbonyl Serine [ZSer] (24) :

To an ice-cooled and well stirred solution of serine (100 mmol) in 25 mL 4N NaOH (100 mmol) was added 30 mL of NaOH (120 mmol) and 18.7 g (100 mmol) of benzyloxycarbonyl chloride, alternately, in about 5 equal parts, over a period of 0.5 h. The reaction mixture was left stirred for 24 h at 0°C, extracted with ether (2 x 30 mL) to remove excess benzyloxycarbonyl chloride, the aqueous layer adjusted to pH 2 with 5N HCl, under cooling in an ice bath, extracted with EtOAc (3 x 30 mL), the organic layers dried ($MgSO_4$), solvents evaporated *in vacuo* and the residue crystallized from EtOAc/hexane to give (24).

yield	:	94%
mp	:	115-116°C (lit.[48] mp 114-116°C)
ir(KBr)ν_{max} cm^{-1}	:	3460, 3320, 2928, 1724, 1665, 1515

XXV. N-Benzyloxycarbonyl Serine 3-Acetyl Tyrosine Methyl Ester [ZSerTyr(3-Ac)OMe] (25) :

DCC coupling of ZSer (24) (0.478 g, 2 mmol) with Tyr(3-Ac)OMe.HCl (2) (0.57 g, 2.08 mmol) by General Procedure-G afforded 0.63 g of ZSerTyr(3-Ac)OMe (25).

yield	:	69%
mp	:	155°C
ir(KBr)ν_{max} cm^{-1}	:	3300, 2926, 1723, 1622, 1511
nmr($CDCl_3$-DMSO-d_6) δ	:	2.59 (s, 3H, $COCH_3$), 3.09 (d, 2H, Tyr $C^{\beta}H_2$), 3.72 (m, 5H, $COOCH_3$ + Ser $C^{\beta}H_2$), 4.22 (m, 1H, Ser $C^{\alpha}H$), 4.78 (q, 1H, Tyr $C^{\alpha}H$), 5.06 (s, 2H, Z CH_2), 6.53 (d, 1H, Ser NH), 6.88 (d, 1H, Tyr C-5 H), 7.19-7.78 (m, 8H, Z aromatic + Tyr C-6 H + Tyr C-2 H + Tyr NH), 12.16 (s, 1H, phenolic OH)
ms (m/z)	:	459 $(MH)^+$
anal	:	Calcd. for $C_{23}H_{26}N_2O_8$ (M.W. 458) C, 60.26; H, 5.67; N, 6.11 % Found : C, 60.14; H, 5.83; N, 5.87 %

XXVI. N-^{t}Butyloxycarbonyl Alanyl 3-Oximinoacetyl Tyrosine Methyl Ester [BocAlaTyr(3-Oximinoacetyl)OMe] (26) :

BocAlaTyr(3-Ac)OMe (16) (0.25 g, 0.61 mmol) by General Procedure-A gave 0.184 g of (26).

yield	:	71%
mp	:	172°C
ir(KBr)ν_{max} cm^{-1}	:	3339, 3258,,2986, 2929, 1742, 1690, 1670, 1623, 1537
nmr($CDCl_3$) δ	:	1.47 (m, 12H, Ala CH_3 + Boc CH_3), 2.28 (s, 3H, oximino CH_3), 3.13 (d, 2H, Tyr $C^{\beta}H_2$), 3.84 (s, 3H, $COOCH_3$), 4.17 (m, 1H, Ala $C^{\alpha}H$), 4.93 (q, 1H, Tyr $C^{\alpha}H$), 5.37 (d, 1H, Tyr NH), 6.47-7.34 (m, 4H, aromatic + Tyr NH), 9.43 (s, 1H, N-OH), 11.74 (s, 1H, phenolic OH)
ms (m/z)	:	424 $(MH)^+$
anal	:	Calcd. for $C_{20}H_{29}N_3O_7$ (M.W. 423) C, 56.73; H, 6.85; N, 9.92 % Found : C, 57.15; H, 6.70; N, 9.94 %

XXVII. N-^{t}Butyloxycarbonyl Alanyl 3-Oximinoacetyl Tyrosine Serine Methyl Ester [BocAlaTyr(3-Oximinoacetyl)SerOMe] (27) :

BocAlaTyr(3-Ac)SerOMe (19) (0.1 g, 0.2 mmol) by General Procedure-A gave 0.086 g of (27).

yield	:	83%
mp	:	104°C
ir(KBr)ν_{max} cm^{-1}	:	3317, 2928, 1743, 1686, 1660, 1521
nmr($CDCl_3$-DMSO-d_6) δ	:	1.28(d, 3H, Ala CH_3), 1.44 (s, 9H, Boc CH_3), 2.31 (s, 3H, oximino CH_3), 3.09 (d, 2H, Tyr $C^{\beta}H_2$), 3.78 (m, 5H, $COOCH_3$ + Ser $C^{\beta}H_2$), 4.09 (m, 1H, Ala $C^{\alpha}H$), 4.63 (m, 2H, Tyr $C^{\alpha}H$ + Ser $C^{\alpha}H$), 5.75 (d, 1H, Ala NH), 6.75-7.68 (m, 5H, aromatic + Tyr NH + Ser NH), 10.8 (s, 1H, N-OH), 11.7 (s, 1H, phenolic OH)
ms (m/z)	:	511 $(MH)^+$

XXVIII. N-Benzyloxycarbonyl Alanyl 3-Oximinoacetyl Tyrosine Methyl Ester [ZAlaTyr(3-Oximinoacetyl)OMe] (28) :

ZAlaTyr(3-Ac)OMe (21) (0.3 g, 0.68 mmol) by General Procedure-A gave 0.219 g of (28).

yield	:	70%
mp	:	165°C
ir(KBr)ν_{max} cm^{-1}	:	3453, 3305, 2929, 1733, 1684, 1650, 1534
nmr($CDCl_3$-DMSO-d_6) δ	:	1.31 (d, 3H, Ala CH_3), 2.31 (s, 3H, oximino CH_3), 3.03 (d, 2H, Tyr $C^{\beta}H_2$), 3.69 (s, 3H, $COOCH_3$), 4.22 (m, 1H, Ala $C^{\alpha}H$), 4.72 (q, 1H, Tyr $C^{\alpha}H$), 5.06 (s, 2H Z CH_2), 6.50 (d, 1H, Ala NH), 6.75 (d, 1H, Tyr C-5 H), 7.03 (d, 1H, Tyr C-6 H), 7.09-7.63 (m, 7H, Tyr C-2 H + Tyr NH + Z aromatic) 11.06 (s, 1H, N-OH), 11.75 (s, 1H, phenolic OH)
ms (m/z)	:	458 $(MH)^+$
anal	:	Calcd. for $C_{23}H_{27}N_3O_7$ (M.W. 457) C, 60.39; H, 5.90; N, 9.19 % Found : C, 59.97; H, 5.83; N, 9.14 %

XXIX. N-Benzyloxycarbonyl Alanyl 3-Oximinoacetyl Tyrosine Serine Methyl Ester [ZAlaTyr(3-Oximinoacetyl)SerOMe (29) :

ZAlaTyr(3-Ac)SerOMe (23) (0.15 g, 0.28 mmol) by General Procedure-A gave 0.128 g of (29).

yield	:	84%
mp	:	204° C
ir(KBr)ν_{max} cm^{-1}	:	3450, 2948, 1720, 1678, 1650
ms (m/z)	:	545 $(MH)^+$
anal	:	Calcd. for $C_{26}H_{32}N_4O_9$ (M.W. 544)
		C, 57.35; H, 5.88; N, 10.29 %
		Found : C, 57.63; H, 5.29; N, 10.53 %

XXX. N-Benzyloxycarbonyl Seryl 3-Oximinoacetyl Tyrosine Methyl Ester [ZSerTyr(3-Oximinoacetyl)OMe] (30) :

ZSerTyr(3-Ac)OMe (25) (0.12 g, 0.26 mmol) by General Procedure-A gave 0.108 g of (30).

yield	:	88%
mp	:	145-147°C
ir(KBr)ν_{max} cm^{-1}	:	3527, 3426, 3324, 2945, 1728, 1692, 1645, 1529
ms (m/z)	:	473 $(MH)^+$
anal	:	Calcd. for $C_{23}H_{27}N_3O_8$ (M.W. 473)
		C, 58.35; H, 5.71; N, 8.87 %
		Found : C, 58.53; H, 5.37; N, 9.13 %

XXXI. N-^{t}Butyloxycarbonyl Alanyl 3-Acetyl Tyrosine Methyl Ester - AEH Schiff Base (31) :

BocAlaTyr(3-Ac)OMe (16) (0.2 g, 0.49 mmol) by General Procedure-B gave 0.15 g of (31).

yield	:	68%
mp	:	163°C
ir(KBr)ν_{max} cm^{-1}	:	3326,2980, 2936, 1744, 1665, 1606, 1560
nmr($CDCl_3$) δ	:	1.31 (m, 12H, Ala CH_3 + Boc CH_3),1.86 (s,s, 6H, AEH CH_3 × 2), 2.28 (s, 3H, CH_3), 3.0 (d, 2H, $C^{\beta}H_2$), 3.5-4.17 (m, 8H, $COOCH_3$ + -CH_2-CH_2- + Ala $C^{\alpha}H$), 4.56-5.16 (m, 3H, Tyr $C^{\alpha}H$ + enolic CH + Ala NH), 6.56 (d, 1H, Tyr NH), 6.72 (d, 1H, Tyr C-5 H), 6.94 (d, 1H, Tyr C-6 H), 7.19 (s, 1H, Tyr C-2 H), 10.81 (s, 1H, enolic OH), 15.15 (s, 1H, phenolic OH)

ms (m/z)	:	533 $(MH)^+$
anal	:	Calcd. for $C_{27}H_{40}N_4O_7$ (M.W. 532) C, 60.90; H, 7.51; N, 10.52 % Found : C, 60.06; H, 7.30; N, 10.40 %

XXXII. N-^{t}Butyloxycarbonyl Alanyl 3-Acetyl Tyrosyl Serine Methyl Ester - AEH Schiff Base (32) :

BocAlaTyr(3-Ac)SerOMe (19) (0.04 g, 0.08 mmol) by General Procedure-B gave 0.036 g of (32).

yield	:	72%
mp	:	164°C
ir(KBr)ν_{max} cm^{-1}	:	3276, 2928, 1743, 1668, 1635, 1537, 1485
nmr($CDCl_3$) δ	:	1.34 (m, 12H, Ala CH_3 + Boc CH_3), 1.93 (s, 6H, AEH CH_3 × 2), 2.62 (s, 3H, CH_3), 3.12 (d, 2H, Tyr $C^{\beta}H_2$), 3.43-4.25 (m, 10H, $COOCH_3$ + -CH_2-CH_2- + Ala $C^{\alpha}H$ + Ser $C^{\beta}H_2$), 4.68 (m, 2H, Tyr $C^{\alpha}H$ + Ser $C^{\alpha}H$), 5.0 (s, 1H, enolic CH), 5.31 (d, 1H, Ala NH), 6.78-7.78 (m, 5H, Tyr NH + Ser NH + aromatic) , 10.93 (s, 1H, enolic OH), 12.15 (s, 1H, phenolic OH)
ms (m/z)	:	620 $(MH)^+$

XXXIII. N-Benzyloxycarbonyl Alanyl 3-Acetyl Tyrosine Methyl Ester - AEH Schiff Base (33) :

ZAlaTyr(3-Ac)OMe (21) (0.25 g, 0.56 mmol) by General Procedure-B gave 0.227 g of (33).

yield	:	71%
mp	:	215°C
ir(KBr)ν_{max} cm^{-1}	:	3310, 3060, 2956, 1740, 1682, 1649, 1610, 1525
nmr($CDCl_3$-DMSO-d_6) δ	:	1.31 (m, 3H, Ala CH_3), 1.97 (s,s, 6H, AEH CH_3 × 2), 2.56 (s, 3H, CH_3), 3.09 (d, 2H, Tyr $C^{\beta}H_2$), 3.72 (m, 7H, $COOCH_3$ + -CH_2-CH_2-), 4.19 (m, 1H, Ala $C^{\alpha}H$), 4.88 (q, 1H, Tyr $C^{\alpha}H$), 5.0 (s, 1H, enolic CH), 5.09 (s, 2H, Z CH_2), 5.56 (d, 1H, Ala NH), 6.75-7.63 (m, 9H, Tyr NH + aromatic), 10.94 (s, 1H, enolic OH)

XXXIV. Bis-N-Benzyloxycarbonyl Alanyl 3-Acetyl Tyrosine Methyl Ester - Ethylenediamine Schiff Base (34) :

ZAlaTyr(3-Ac)OMe (21) (0.08 g, 0.18 mmol) by General Procedure-C gave 0.072 g of (34).

yield	:	88%
mp	:	213-215°C
ir(KBr)ν_{max} cm^{-1}	:	3302, 1717, 1647, 1608, 1522
ms (m/z)	:	909 $(MH)^+$
anal	:	Calcd. for $C_{48}H_{56}N_6O_{12}$ (M.W. 908)
		C, 63.43; H, 6.16; N, 9.25 %
		Found : C, 63.88; H, 6.43; N, 9.48 %

XXXV. Bis-N-Benzyloxycarbonyl Alanyl 3-Acetyl Tyrosyl Serine Methyl Ester - Ethylenediamine Schiff Base (35) :

ZAlaTyr(3-Ac)SerOMe (23) (0.052 g, 0.1 mmol) by General Procedure-C gave 0.047 g of (35).

yield	:	89%
mp	:	249-251°C
ir(KBr)ν_{max} cm^{-1}	:	3286, 1740, 1683, 1628, 1528
ms (m/z)	:	1083 $(MH)^+$
anal	:	Calcd. for $C_{54}H_{66}N_8O_{16}$ (M.W. 1082)
		C, 59.88; H, 6.09; N, 10.35 %
		Found : C, 60.32; H, 6.14; N, 10.88 %

XXXVI. Bis-N-Benzyloxycarbonyl Seryl 3-Acetyl Tyrosine Methyl Ester - Ethylenediamine Schiff Base (36) :

ZSerTyr(3-Ac)OMe (25) (0.1 g, 0.218 mmol) by General Procedure-C gave 0.092 g of (36).

yield	:	90%
mp	:	155-158°C

ir(KBr)ν_{max} cm^{-1}	:	3295, 2927, 1723, 1680, 1645, 1606, 1524
ms (m/z)	:	941 $(MH)^+$
anal	:	Calcd. for $C_{48}H_{56}N_6O_{14}$ (M.W. 940)
		C, 61.27; H, 5.95; N, 8.93 %
		Found : C, 61.43; H, 5.84; N, 9.23 %

XXXVII. Copper Complex of BzTyr(3-Oximinoacetyl)OMe (37) :

BzTyr(3-Oximinoacetyl)OMe (7) (0.059 g, 0.16 mmol) on treatment with $Cu(OAc)_2$.H_2O (0.015 g, 0.08 mmol) afforded 0.05 g of (37), (General Procedure-D).

yield	:	78%
mp	:	253°C
ir(KBr)ν_{max} cm^{-1}	:	3289, 2951, 1741, 1637, 1780, 1531
epr($CHCl_3$, rt)	:	$A_{\|\|} = 95$, $g_{\|\|} = 2.112$, $g_{\perp} = 2.013$
ms (m/z)	:	774 $(MH)^+$
uv-vis	:	259, 345 647
($CHCl_3$)λ_{max} nm		
anal	:	Calcd. for $C_{38}H_{38}N_4O_{10}$ Cu (M.W. 773)
		C, 58.95; H, 4.91; N, 7.23 %
		Found : C, 58.73; H, 5.11; N, 7.22 %

XXXVIII. Nickel Complex of BzTyr(3-Oximinoacetyl)OMe (38) :

BzTyr(3-Oximinoacetyl)OMe (7) (0.059 g, 0.16 mmol) on treatment with $Ni(OAc)_2$.-$4H_2O$ (0.02 g, 0.08 mmol) afforded 0.052 g of (38), (General Procedure-D).

yield	:	83%
mp	:	265°C
ir(KBr)ν_{max} cm^{-1}	:	3287, 3027, 2923, 1740, 1636
nmr($CDCl_3$) δ	:	2.31 (s, 6H, CH_3 × 2), 3.15 (d, 4H, $C^{\beta}H_2$ × 2), 3.75 (s, 6H, $COOCH_3$ × 2), 5.0 (q, 2H, $C^{\alpha}H$ × 2), 6.56 (d, 2H, NH × 2), 6.69-7.93 (m, 16H, aromatic), 10.87 (s, 2H, N-OH × 2)
ms (m/z)	:	769 $(MH)^+$
uv-vis	:	259, 301, 382 602
($CHCl_3$)λ_{max} nm		

XXXIX. Cobalt Complex of BzTyr(3-Oximinoacetyl)OMe (39) :

BzTyr(3-Oximinoacetyl)OMe (7) (0.075 g, 0.21 mmol) on treatment with $Co(OAc)_2.4H_2O$ (0.027 g, 0.11 mmol) afforded 0.069 g of (39), (General Procedure-D).

yield	:	85%
mp	:	151°C (dec.)
ir(KBr)ν_{max} cm^{-1}	:	3314, 1717, 1646, 1540, 1492
ms (m/z)	:	770 $(MH)^+$
uv-vis $(CHCl_3)\lambda_{max}$ nm	:	255, 311 650

XL. Copper Complex of ZTyr(3-Oximinoacetyl)OMe (40) :

ZTyr(3-Oximinoacetyl)OMe (8) (0.066 g, 0.17 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.017 g, 0.08 mmol) afforded 0.052 g of (40), (General Procedure-D).

yield	:	73%
mp	:	208°C
ir(KBr)ν_{max} cm^{-1}	:	3318, 2950, 1740, 1690, 1636, 1479
epr($CHCl_3$, rt)	:	$A_{\|\|} = 100$, $g_{\|\|} = 2.115$, $g_{\perp} = 2.005$
ms (m/z)	:	896, 835 $(MH)^+$
uv-vis $(DMSO)\lambda_{max}$ nm	:	259, 419, 646

XLI. Nickel Complex of ZTyr(3-Oximinoacetyl)OMe (41) :

ZTyr(3-Oximinoacetyl)OMe (8) (0.066 g, 0.17 mmol) on treatment with $Ni(OAc)_2.4H_2O$ (0.019 g, 0.08 mmol) afforded 0.053 g of (41), (General Procedure-D).

yield	:	75%
mp	:	213-215°C
ir(KBr)ν_{max} cm^{-1}	:	3324, 2921, 1737, 1688, 1632, 1528
ms (m/z)	:	829 $(MH)^+$
uv-vis $(DMSO)\lambda_{max}$ nm	:	260, 305, 392, 601

XLII. Copper Complex of AcTyr(3-Oximinoacetyl)OMe (42) :

AcTyr(3-Oximinoacetyl)OMe (9) (0.055 g, 0.19 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.018 g, 0.09 mmol) afforded 0.047 g of (42), (General Procedure-D).

yield	:	76%
mp	:	270-272°C
ir(KBr)ν_{max} cm^{-1}	:	3304, 2924, 1741, 1654, 1545, 1479
epr($CHCl_3$, rt)	:	$A_{\|\|} = 100$, $g_{\|\|} = 2.114$, $g_{\perp} = 2.013$
ms (m/z)	:	651 $(MH)^+$
uv-vis (DMSO)λ_{max} nm	:	269, 390, 584 269, 390, 584

XLIII. Nickel Complex of AcTyr(3-Oximinoacetyl)OMe (43) :

AcTyr(3-Oximinoacetyl)OMe (9) (0.043 g, 0.15 mmol) on treatment with $Ni(OAc)_2.4H_2O$ (0.018 g, 0.073 mmol) afforded 0.039 g of (43), (General Procedure-D).

yield	:	81%
mp	:	292-295°C
ir(KBr)ν_{max} cm^{-1}	:	3448, 3292, 1736, 1652, 1552, 1507
ms (m/z)	:	645 $(MH)^+$
uv-vis (DMSO)λ_{max} nm	:	262, 304, 366, 468(sh), 599

XLIV. Copper Complex of BzTyr(3-Ac)OMe - AEH Schiff Base (44) :

The AEH Schiff base (12) (0.08 g, 0.17 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.032 g, 0.17 mmol) afforded 0.067 g of (44), (General Procedure-E).

yield	:	74%
mp	:	115-118°C
ir(KBr)ν_{max} cm^{-1}	:	3447, 3061, 2949, 1735, 1641, 1592, 1514
epr(MeOH, rt)	:	$A_{\|\|} = 90$, $g_{\|\|} = 2.111$, $g_{\perp} = 2.016$
(MeOH, -196°C)	:	$A_{\|\|} = 185$, $g_1 = 2.359$, $g_2 = 2.087$, $g_3 = 1.999$
ms (m/z)	:	527 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm	:	246, 328, 387, 546.

anal : Calcd. for $C_{26}H_{29}N_3O_5$ (M.W. 526)
C, 59.25; H, 5.50; N, 7.97 %
Found : C, 56.19; H, 5.62; N, 7.55 %

XLV. Nickel Complex of BzTyr(3-Ac)OMe - AEH Schiff Base (45) :

The AEH Schiff base (12) (0.08 g, 0.17 mmol) on treatment with $Ni(OAc)_2.4H_2O$ (0.042 g, 0.17 mmol) afforded 0.071 g of (45), (General Procedure-E).

yield : 80%
mp : 130°C
ir(KBr)ν_{max} cm^{-1} : 3421, 2950, 1741, 1638 1615, 1578, 1517
nmr($CDCl_3$) δ : 1.88 (s,s, 6H, AEH $CH_3 \times 2$), 2.19 (s, 3H, CH_3), 2.97-3.53 (m, 6H, -CH_2-CH_2 + $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.97 (s, 1H, =C-H), 5.0 (q, 2H, $C^{\alpha}H$), 6.63 (d, 1H, NH), 6.81-7.88 (m, 8H, aromatic)
ms (m/z) : 521 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm : 250, 333, 412 562

XLVI. Cobalt Complex of BzTyr(3-Ac)OMe - AEH Schiff Base (46) :

The AEH Schiff base (12) (0.03 g, 0.064 mmol) on treatment with $Co(OAc)_2.4H_2O$ (0.016 g, 0.064 mmol) gave 0.024 g of (46), (General Procedure-E).

yield : 71%
mp : 168°C
ms (m/z) : 522 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm : 241, 389(sh), 761
ir(KBr)ν_{max} cm^{-1} : 3398, 2925, 1741, 1616, 1560

XLVII. Copper Complex of Bis-BzTyr(3-AC)OMe - Ethylenediamine Schiff Base (47) :

The EDA Schiff base (13) (0.05 g, 0.07 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.014 g, 0.07 mmol) gave 0.042 g of (47), (General Procedure-F).

yield	:	78%
mp	:	148-149°C
ir(KBr)ν_{max} cm^{-1}	:	3378, 2951, 1740, 1646, 1615, 1586, 1530.
epr($CHCl_3$, rt)	:	$A_{\|\|} = 100$, $g_{\|\|} = 2.112$, $g_{\perp} = 2.011$
($CHCl_3$, -196°C)	:	$A_{\|\|} = 205$, $g_1 = 2.193$, $g_2 = 2.053$, $g_3 = 1.980$
ms (m/z)	:	769 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm	:	245, 371, 550

XLVIII. Nickel Complex of Bis-BzTyr(3-Ac)OMe - Ethylenediamine Schiff Base (48) :

The EDA Schiff base (13) (0.05 g, 0.07 mmol) on treatment with $Ni(OAc)_2.4H_2O$ (0.018 g, 0.07 mmol) gave 0.043 g of (48), (General Procedure-F).

yield	:	80%
mp	:	206-208°C
ir(KBr)ν_{max} cm^{-1}	:	3431, 2952, 1734, 1646, 1615, 1578, 1531
nmr($CDCl_3$) δ	:	2.0 (s, 6H, CH_3 × 2), 3.13 (d, 4H, $C^{\beta}H_2$ × 2), 3.47 (m, 4H, -CH_2-CH_2), 3.66 (s, 6H, $COOCH_3$ × 2), 5.03 (q, 2H, $C^{\alpha}H$ × 2), 6.66-7.94 (m, 18H, NH × 2 + aromatic)
ms (m/z)	:	763 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm	:	260, 339, 415 560

XLIX. Cobalt Complex of Bis-BzTyr(3-Ac)OMe - Ethylenediamine Schiff Base (49) :

The EDA Schiff base (13) (0.05 g, 0.07 mmol) on treatment with $Co(OAc)_2.4H_2O$ (0.018 g, 0.07 mmol) gave 0.039 g of (49), (General Procedure-F).

yield	:	72%
mp	:	165°C
ir(KBr)ν_{max} cm^{-1}	:	3352, 2926, 1738, 1648, 1615, 1540, 1484
ms (m/z)	:	764 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max} nm	:	246, 365, 635

L. Copper Complex of Bis-ZTyr(3-Ac)OMe - Ethylenediamine Schiff Base (50) :

The EDA Schiff base (14) (0.05 g, 0.065 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.013 g, 0.065 mmol) gave 0.04 g of (50), (General Procedure-F).

yield	:	74%
mp	:	115°C
ir(KBr)ν_{max} cm^{-1}	:	3329, 2950, 1720, 1614, 1587, 1529
epr($CHCl_3$, rt)	:	$A_{\|\|} = 95$, $g_{\|\|} = 2.111$, $g_{\perp} = 2.013$
($CHCl_3$ -196°C)	:	$A_{\|\|} = 215$, $g_1 = 2.191$, $g_2 = 2.042$, $g_3 = 1.978$
uv-vis (DMSO)λ_{max}nm	:	272, 373, 559

LI. Ni Complex of Bis-ZTyr(3-Ac)OMe - Ethylenediamine Schiff Base(51) :

The EDA Schiff base (14) (0.05 g, 0.065 mmol) on treatment with $Ni(OAc)_2.4H_2O$ (0.016 g, 0.065 mmol) gave 0.042 g of (51), (General Procedure-F).

yield	:	78%
mp	:	190-195°C
ir(KBr)ν_{max} cm^{-1}	:	3346, 2923, 1718, 1613, 1578, 1529
nmr($CDCl_3$) δ	:	1.75 (s, 6H, CH_3 x 2), 2.88 (br, 4H, $C^{\beta}H_2$ x 2), 3.50 (s, 4H, -CH_2CH_2-), 3.72 (s, 6H, $COOCH_3$ x 2), 4.47 (br, 2H, $C^{\alpha}H$ x 2), 5.06 (s, 4H, Z CH_2 x 2), 5.47 (brd, 2H, NH x 2), 6.91 (m, 6H, Tyr aromatic), 7.34 (s, 10H, Z aromatic)
uv-vis (DMSO)λ_{max}nm	:	263, 337, 410, 557

LII. Cobalt Complex of Bis-ZTyr(3-Ac)OMe - Ethylenediamine Schiff Base (52) :

The EDA Schiff base (14) (0.05 g, 0.065 mmol) on treatment with $Co(OAc)_2.4H_2O$ (0.016 g, 0.065 mmol) gave 0.04 g of (52), (General Procedure-F).

yield	:	75%
mp	:	157-162°C
ir(KBr)ν_{max} cm^{-1}	:	3413, 2928, 1720, 1616, 1561, 1481
uv-vis (DMSO)λ_{max}nm	:	263, 375, 554

LIII. Copper Complex of BocAlaTyr(3-Oximinoacetyl)OMe (53) :

BocAlaTyr(3-Oximinoacetyl)OMe (26) (0.15 g, 0.35 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.034 g, 0.175 mmol) gave 0.103 g of (53), (General Procedure-D).

yield	:	67%
mp	:	226°C
ir(KBr)ν_{max} cm^{-1}	:	3387, 3329, 2977, 1741, 1666, 1520
epr($CHCl_3$, rt)	:	$A_{\|\|} = 100$, $g_{\|\|} = 2.116$, $g_{\perp} = 2.016$
uv-vis ($CHCl_3$)λ_{max}nm	:	254, 345, 648
anal	:	Calcd. for $C_{40}H_{56}N_6O_{14}Cu$ (M.W. 907) C, 52.89; H, 6.17; N, 9.25 % Found : C, 52.64; H, 6.25; N, 9.14 %

LIV. Nickel Complex of BocAlaTyr(3-Oximinoacetyl)OMe (54) :

BocAlaTyr(3-Oximinoacetyl)OMe (26) (0.075 g, 0.177 mmol) on treatment with $Ni(OAc)_2.4H_2O$ (0.021 g, 0.088 mmol) gave 0.055 g of (54), (General Procedure-D).

yield	:	69%
mp	:	216-217°C
ir(KBr)ν_{max} cm^{-1}	:	3327, 2977, 1740, 1667, 1613, 1522
nmr($CDCl_3$) δ	:	1.34 (m, 24H, Boc CH_3 x 6 + Ala CH_3 x 2), 2.38 (s, 6H, CH_3 x 2), 3.0 (d, 4H, Tyr $C^{\beta}H_2$ x 2), 3.69 (s, 6H, $COOCH_3$ x 2), 4.06 (m, 2H, Ala $C^{\alpha}H$ x 2), 4.72 (m, 4H, Tyr $C^{\alpha}H$ x 2 + Ala NH x 2), 6.47 (d, 2H, Tyr NH x 2), 6.53-7.31 (m, 6H, aromatic), 10.84 (s, 2H, N-OH x 2)
ms (m/z)	:	902 $(MH)^+$
uv-vis ($CHCl_3$)λ_{max}nm	:	256, 301, 381, 602

LV. Cobalt Complex of BocAlaTyr(3-Oximinoacetyl)OMe (55) :

BocAlaTyr(3-Oximinoacetyl)OMe (26) (0.05 g, 0.11 mmol) on treatment with $Co(OAc)_2.4H_2O$ (0.014 g, 0.055 mmol) gave 0.038 g of (55), (General Procedure-D).

yield	:	72%
mp	:	157°C (dec.)
ir(KBr)ν_{max} cm^{-1}	:	3341, 2929, 1742, 1691, 1670, 1539, 1510
ms (m/z)	:	903 $(M)^+$
uv-vis $(CHCl_3)\lambda_{max}$nm	:	252, 300(sh), 659(sh)
anal	:	Calcd. for $C_{40}H_{56}N_6O_{14}Co$ (M.W. 903)
		C, 53.15; H, 6.20; N, 9.30 %
		Found : C, 53.44; H, 6.50; N, 9.06 %

LVI. Copper Complex of BocAlaTyr(3-Oximinoacetyl)SerOMe (56) :

BocAlaTyr(3-Oximinoacetyl)SerOMe (27) (0.03 g, 0.058 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.006 g, 0.029 mmol) gave 0.026 g of (56), (General Procedure-D).

yield	:	84%
mp	:	255°C (dec.)
ir(KBr)ν_{max} cm^{-1}	:	3315, 2977, 2929, 1744, 1652, 1510
epr(DMSO, -196°C)	:	$A_{\|\|} = 195$, $g_1 = 2.236$, $g_2 = 2.085$, $g_3 = 2.016$
uv-vis (DMSO)λ_{max} nm	:	263, 342, 631 263, 342, 631

LVII. Nickel Complex of BocAlaTyr(3-Oximinoacetyl)SerOMe (57) :

BocAlaTyr(3-Oximinoacetyl)SerOMe (27) (0.024 g, 0.048 mmol) on treatment with $Ni(OAc)_2.4H_2O$ (0.006 g, 0.024 mmol) gave 0.018 g of (57), (General Procedure-D).

yield	:	72%
mp	:	252°C
ir(KBr)ν_{max} cm^{-1}	:	3419, 2925, 2854, 1741, 1652, 1508
ms (m/z)	:	1076 $(M)^+$

LVIII. Cobalt Complex of BocAlaTyr(3-Oximinoacetyl)SerOMe (58) :

BocAlaTyr(3-Oximinoacetyl)SerOMe (27) (0.024 g, 0.048 mmol) on treatment with $Co(OAc)_2.4H_2O$ (0.006 g, 0.024 mmol) gave 0.017 g of (58), (General Procedure-D).

yield	:	68%
mp	:	>300°C
ir(KBr)ν_{max} cm^{-1}	:	3416, 2976, 2923, 1717, 1684, 1654, 1558, 1540
ms (m/z)	:	1077 $(M)^+$

LIX. Copper Complex of ZAlaTyr(3-Oximinoacetyl)OMe (59) :

ZAlaTyr(3-Oximinoacetyl)OMe (28) (0.05 g, 0.11 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.011 g, 0.055 mmol) afforded 0.043 g of (59), (General Procedure-D).

yield	:	80%
mp	:	225-228°C
ir(KBr)ν_{max} cm^{-1}	:	3298, 2942, 1722, 1680, 1635, 1522
epr (DMF, -196°C)	:	$A_{\|\|} = 195$, $g_1 = 2.079$, $g_2 = 2.053$, $g_3 = 2.001$
uv-vis (DMSO)λ_{max}nm	:	267, 344, 386, 594
anal	:	Calcd. for $C_{46}H_{52}N_6O_{14}Cu$ (M.W. 975) C, 56.58; H, 5.33; N, 8.61 % Found : C, 56.44; H, 5.03; N, 9.17 %

LX. Nickel Complex of ZAlaTyr(3-Oximinoacetyl)OMe (60) :

ZAlaTyr(3-Oximinoacetyl)OMe (28) (0.07 g, 0.15 mmol) on treatment with $Ni(OAc)_2.4H_2O$ (0.019 g, 0.076 mmol) afforded 0.057 g of (60), (General Procedure-D).

yield	:	78%
mp	:	230-233°C
ir(KBr)ν_{max} cm^{-1}	:	3302, 3033, 2927, 1740, 1689, 1651, 1540
ms (m/z)	:	972 $(MH)^+$
uv-vis (DMSO)λ_{max}nm	:	266, 300, 373, 598

LXI. Copper Complex of ZAlaTyr(3-Oximinoacetyl)SerOMe (61) :

ZAlaTyr(3-Oximinoacetyl)SerOMe (29) (0.05 g, 0.092 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.009 g, 0.046 mmol) afforded 0.034 g of (61), (General Procedure-D).

yield	:	64%
mp	:	245-247°C
ir(KBr)ν_{max} cm^{-1}	:	3288, 3064, 2928, 1744, 1694, 1646, 1541
epr(DMSO, -196°C)	:	$A_{\|\|} = 195$, $g_1 = 2.220$, $g_2 = 2.055$, $g_3 = 2.004$
uv-vis (DMSO)λ_{max}nm	:	260, 358, 423(sh), 654

LXII. Copper Complex of ZSerTyr(3-Oximinoacetyl)OMe (62) :

ZSerTyr(3-Oximinoacetyl)OMe (30) (0.06 g, 0.13 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.013 g, 0.063 mmol) afforded 0.054 g of (62), (General Procedure-D).

yield	:	82%
mp	:	198-202°C
ir(KBr)ν_{max} cm^{-1}	:	3302, 3068, 2947, 1719, 1640, 1605, 1530
epr(DMSO, -196°C)	:	$A_{\|\|} = 190$, $g_1 = 2.225$, $g_2 = 2.056$, $g_3 = 2.004$
uv-vis (DMSO)λ_{max}nm	:	263, 344, 380, 629
anal		Calcd. for $C_{46}H_{52}N_6O_{16}Cu$ (M.W. 1007)
		C, 54.78; H, 5.16; N, 8.33 %
		Found : C, 54.48; H, 5.18; N, 8.38 %

LXIII. Nickel Complex of ZSerTyr(3-Oximinoacetyl)OMe (63) :

ZSerTyr(3-Oximinoacetyl)OMe (30) (0.03 g, 0.06 mmol) on treatment with $Ni(OAc)_2.4H_2O$ (0.008 g, 0.03 mmol) afforded 0.022 g of (63), (General Procedure-D).

yield	:	73%
mp	:	228-230°C
ir(KBr)ν_{max} cm^{-1}	:	3302, 2926, 1735, 1695, 1652, 1541
ms (m/z)	:	1004 $(MH)^+$
uv-vis (DMSO)λ_{max} nm	:	306, 374, 599

LXIV. Copper Complex of BocAlaTyr(3-Ac)OMe - AEH Schiff Base (64) :

The AEH Schiff base (31) (0.023 g, 0.043 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.009 g, 0.043 mmol) afforded 0.017 g of (64), (General Procedure-E).

yield	:	68%
mp	:	125-130°C
ir(KBr)ν_{max} cm^{-1}	:	3300, 1730, 1650, 1580, 1500
epr (MeOH, rt)	:	$A_{\|\|} = 195$, $g_{\|\|} = 2.110$, $g_{\perp} = 2.017$
(MeOH, -196°C)	:	$A_{\|\|} = 195$, $g_1 = 2.207$, $g_2 = 2.042$, $g_3 = 1.990$
ms (m/z)	:	594 $(MH)^+$
uv-vis $(CHCl_3)\lambda_{max}$ nm	:	274, 313, 382(sh), 547, 650(sh)
anal	:	Calcd. for $C_{27}H_{38}N_4O_7Cu$ (M.W. 593) C, 54.59; H, 6.40; N, 9.43 % Found : 55.03; H, 6.36; N, 9.31 %

LXV. Copper Complex of BocAlaTyr(3-Ac)SerOMe - AEH Schiff Base (65):

The AEH Schiff base (32) (0.04 g, 0.064 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.014 g, 0.064 mmol) afforded 0.032 g of (65), (General Procedure-E).

yield	:	73%
mp	:	142-150°C
ir(KBr)ν_{max} cm^{-1}	:	3410, 3290, 1730, 1632, 1580, 1503
epr ($CHCl_3$, rt)	:	$A_{\|\|} = 90$, $g_{\|\|} = 2.109$, $g_{\perp} = 2.009$
($CHCl_3$, -196°C)	:	$A_{\|\|} = 205$, $g_1 = 2.186$, $g_2 = 2.035$, $g_3 = 1.983$

LXVI. Copper Complex of Bis-ZAlaTyr(3-Ac)OMe - Ethylenediamine Schiff Base (66) :

The EDA Schiff base (34) (0.044 g, 0.048 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.01 g, 0.048 mmol) afforded 0.036 g of (66), (General Procedure-F).

yield	:	78%
mp	:	115-119°C
ir(KBr)ν_{max} cm^{-1}	:	3321, 2927, 1718, 1662, 1614, 1587, 1530
epr ($CHCl_3$, -196°C)	:	$A_{\|\|} = 205$, $g_1 = 2.198$, $g_2 = 2.045$, $g_3 = 1.992$
ms (m/z)	:	971 $(MH)^+$

LXVII. Nickel Complex of Bis-ZAlaTyr(3-Ac)OMe - Ethylenediamine Schiff Base (67) :

The EDA Schiff base (34) (0.044 g, 0.048 mmol) on treatment with $Ni(OAc)_2.4H_2O$ (0.012 g, 0.048 mmol) afforded 0.036 g of (67), (General Procedure-F).

yield	:	78%
mp	:	221-223°C (dec.)
ir(KBr)ν_{max} cm^{-1}	:	3313, 2925, 1741, 1693, 1654, 1616 1579, 1531

LXVIII. Copper complex of Bis-ZSerTyr(3-Ac)OMe - Ethylenediamine Schiff Base (68) :

The EDA Schiff base (36) (0.032 g, 0.034 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.007 g, 0.034 mmol) afforded 0.028 g of (68), (General Procedure-F).

yield	:	82%
mp	:	168-169°C
ir(KBr)ν_{max} cm^{-1}	:	3326, 2928, 2850, 1742, 1626, 1575
epr($CHCl_3$, -196°C)	:	$A_{\|\|} = 200$, $g_1 = 2.198$, $g_2 = 2.066$, $g_3 = 1.986$

LXIX. Nickel Complex of Bis-ZSerTyr(3-Ac)OMe - Ethylenediamine Schiff Base(69) :

The EDA Schiff base (36) (0.05 g, 0.054 mmol) on treatment with $Ni(OAc)_2.4H_2O$ (0.014 g, 0.054 mmol) afforded 0.038 g of (69), (General Procedure-F).

yield : 71%
mp : 139°C
ir(KBr)ν_{max} cm^{-1} : 3313, 2926, 1734, 1654, 1615, 1531
ms (m/z) : 998 $(MH)^+$

LXX. Cobalt complex of Bis-ZSerTyr(3-Ac)OMe - Ethylenediamine Schiff Base (70) :

The EDA Schiff base (36) (0.04 g, 0.043 mmol) on treatment with $Co(OAc)_2.4H_2O$ (0.01 g, 0.043 mmol) afforded 0.032 g of (70), (General Procedure-F).

yield : 76%
mp : 126-129°C
ir(KBr)ν_{max} cm^{-1} : 3312, 2928, 1748, 1690, 1648, 1540
uv-vis (DMSO)λ_{max} nm : 260, 326, 403, 556

LXXI. Copper Complex of Bis-ZAlaTyr(3-Ac)SerOMe - Ethylenediamine Schiff Base(71) :

The EDA Schiff base (35) (0.044 g, 0.04 mmol) on treatment with $Cu(OAc)_2.H_2O$ (0.008 g, 0.04 mmol) afforded 0.04 g of (71), (General Procedure-F).

yield : 87%
mp : 219-220°C
ir(KBr)ν_{max} cm^{-1} : 3303, 2926, 1744, 1653, 1588, 1532
epr ($CHCl_3$, -196°C) : $A_{\|} = 200$, $g_1 = 2.205$, $g_2 = 2.043$, $g_3 = 1.992$
ms (m/z) : 1167 $(MH+Na)^+$, 1145 $(MH)^+$
uv-vis (DMSO)λ_{max} nm : 262, 368, 559

LXXII. Salicylaldehyde - AEH Schiff Base (73) :

The condensation of salicylaldehyde (0.366 g, 3 mmol) with AEH (0.426 g, 3 mmol) provided 0.585 g of (73), (General Procedure-B). The product was purified by column chromatography. (PhH-EtOAc)

yield	:	74%
mp	:	231°C (lit[56]. mp 233-235°C)
ir(KBr)ν_{max} cm^{-1}	:	3253, 2924, 1694, 1634, 1608, 1581, 1552, 1510, 1336

LXXVI. 3-Nitro Tyrosine Methyl Ester Hydrochloride [Tyr(3-Nitro)OMe.-HCl] (77) :

To stirred and ice-cooled dry MeOH (10 mL), was added in drops, $SOCl_2$ (0.5 mL, 7.25 mmol) followed by (76) (1g, 4.4 mmol). Stirring was continued for 2 h and the reaction mixture was allowed to attain room temperature, refluxed for 0.5 h, solvents evaporated and the residue on crystallization from dry MeOH-Et_2O gave 0.827 g of (77).

yield	:	68%
mp	:	188°C (lit.[57] mp 197°C)
ir(KBr)ν_{max} cm^{-1}	:	3198, 2855, 1746, 1635, 1588, 1545, 1319
nmr(D_2O) δ	:	3.25 (d, 2H, $C^{\beta}H_2$, 3.84 (s, 3H, $COOCH_3$, 4.19 (t, 1H, $C^{\alpha}H$, 7.19 (d, 1H, aromatic), 7.53 (d, 1H, aromatic) 8.0 (s, 1H, aromatic)

LXXVII. N-^{t}Butyloxycarbonyl 3-Nitro Tyrosine Methyl Ester [BocTyr(3-Nitro)OMe] (78) :

To a stirred solution of 3-nitro tyrosine methyl ester (1 g, 4.16 mmol) - which was prepared by dropwise addition of 25% ammonia solution to an aqueous suspension of 3-nitro methyl ester hydrochloride (77), in a mixture of DMSO (15 mL) and pyridine (5 mL) - was added t-butylazidoformate (1.2 g, 6.63 mmol) at room temperature. After stirring for 48 h, the solution was concentrated *in vacuo* to $\sim$ 10 mL. EtOAc (15 mL) and water (15 mL) were added to this and the two layers separated. The aqueous layer was extracted with EtOAc (3 x 15 mL), and the combined organic layers washed with 20% citric acid (2 x 8 mL) and water (2 x 10 mL), dried and evaporated to give (78) (1.1 g) as fine solid, which was recrystallized from MeOH.

yield	:	78%
mp	:	94-95°C (lit.[57] mp 98-100°C)
ir(KBr)ν_{max} cm^{-1}	:	3333, 2982, 1733, 1684, 1629, 1576, 1531, 1324
nmr($CDCl_3$) δ	:	1.47 (s, 9H, Boc CH_3 x 3), 3.16 (t, 2H, $C^{\beta}H_2$), 3.81 (s, 3H, $COOCH_3$), 4.63 (q, 1H, $C^{\alpha}H$), 5.09 (d, 1H, NH), 7.16 (d, 1H, aromatic), 7.44 (d, 1H, aromatic), 7.94 (s, 1H, aromatic), 10.56 (s, 1H, phenolic OH)
ms (m/z)	:	341 $(MH)^+$

LXXVIII. 3,4-Dihydroxyphenylalanine Methyl Ester Hydrochloride [DOPA-OMe.HCl] (79) :

To a stirred and ice-cooled dry MeOH (25 mL), was added, in drops, $SOCl_2$ (2.5 mL, 36.25 mmol) followed by L-DOPA (5 g, 25 mmol). The reaction mixture was allowed to attain room temperature and refluxed for 10 h, solvents evaporated *under vacuo* and the residue on crystallization from dry EtOH-Et_2O gave 5.3 g of (79).

yield	:	85%
mp	:	174 °C (lit.[58] mp 170-171°C)
ir(KBr)ν_{max} cm^{-1}	:	3406(br), 1742, 1611, 1528, 1446
nmr(D_2O) δ	:	3.06 (d, 2H, $C^{\beta}H_2$), 3.72 (s, 3H, $COOCH_3$), 4.25 (t, 1H, $C^{\alpha}H$), 6.69 (m, 3H, aromatic)

LXXIX. N-Formyl 3,4-Dihydroxyphenylalanine Methyl Ester [N-Formyl-DOPA-OMe] (80) :

To a stirred and ice-cooled mixture of L-DOPA-OMe.HCl (1.24 g, 5 mmol) and sodium formate (0.374 g, 5.5 mmol) in formic acid (12.5 mL) was added acetic anhydride (5 mL). After stirring for 3 h at room temperature, MeOH (8 mL) was added with ice-cooling and stirring continued for 1 h. The reaction mixture was evaporated, the residue dissolved in EtOAc (45 mL) and filtered . The filtrate was washed with 10% HCl (8 mL), saturated $NaHCO_3$ solution and saturated NaCl solution (8 mL), dried and evaporated. The residue on crystallization from EtOAc-hexane gave 1.14 g of (80) as a white solid.

yield	:	95%
mp	:	123°C (lit.[8] mp 119-121°C)
ir(KBr)ν_{max} cm^{-1}	:	3526, 3348, 1728, 1632, 1533, 1443
nmr($CDCl_3$-DMSO-d_6) δ	:	3.0 (d, 2H, $C^{\beta}H_2$), 3.72 (s, 3H, $COOCH_3$), 4.81 (q, 1H, $C^{\alpha}H$), 6.38-6.88 (m, 3H, aromatic), 7.06 (d, 1H, NH), 8.16 (s, 1H, CHO)
ms (m/z)	:	240 $(MH)^+$
anal	:	Calcd. for $C_{11}H_{13}NO_5$ (M.W. 239)
		C, 55.23; H, 5.44; N, 5.85 %
		Found : C, 54.36; H, 5.08; N, 5.64 %

LXXX. N-Benzyloxycarbonyl 3,4-Dihydroxyphenylalanine Methyl Ester [Z-DOPA-OMe] (81) :

3,4-Dihydroxyphenylalanine methyl ester hydrochloride (79) (0.74 g, 3 mmol) in a two phase mixture of water (15 mL) and $CHCl_3$ (12 mL) at 25°C was heated with Na_2CO_3 (1.27 g, 12 mmol) and benzyloxycarbonylchloride (95%, 0.43 mL, 3 mmol) and the resulting mixture was stirred for 1 h. The $CHCl_3$ layer was separated, washed with saturated NaCl solution, dried, evaporated *in vacuo* and the residue chromatographed on silica gel column (EtOAc:PhH :: 1:4) to give 0.835 g of (81) as a thick syrup.

yield	:	81%
mp	:	gummy
ir(neat)ν_{max} cm^{-1}	:	3392 2956, 1714, 1610, 1520, 1445
nmr($CDCl_3$) δ	:	3.06 (d, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.66 (q, 1H, $C^{\alpha}H$), 5.09 (s, 2H, Z CH_2), 5.44 (d, 1H, NH), 6.47-6.88 (m, 3H, dopa aromatic), 7.41 (s, 5H, Z aromatic)

LXXXI. N-Benzoyl 3,4-Dihydroxyphenylalanine Methyl Ester [Bz-DOPA-OMe] (82) :

3,4-Dihydroxyphenylalanine methyl ester hydrochloride (79) (1.48 g, 6 mmol) in a two phase mixture of water (30 mL) and $CHCl_3$ (25 mL) at 25°C was treated with Na_2CO_3 (2.54 g, 24 mmol) and benzoyl chloride (0.7 mL, 6 mmol) and the resulting mixture was stirred for 1 h. The $CHCl_3$ layer was separated, washed with saturated

NaCl solution, dried ($MgSO_4$), evaporated *in vacuo* and the residue purified by column chromatography on silica gel (EtOAc:PhH :: 1:4) to give 1.52 g of (82).

yield	:	81%
mp	:	72°C
ir(KBr)ν_{max} cm^{-1}	:	3340(br), 1720, 1628, 1586, 1562, 1505, 1472
nmr($CDCl_3$) δ	:	3.03 (d, 2H, $C^{\beta}H_2$), 3.66 (s, 3H, $COOCH_3$), 4.94 (q, 1H, $C^{\alpha}H$), 6.34-6.91 (m, 4H, NH + dopa aromatic), 7.22-7.78 (m, 5H, Bz aromatic)

LXXXII. N,O,O'- Triacetyl 3,4-Dihydroxyphenylalanine Methyl Ester (83) :

To a stirred suspension of DOPA-OMe.HCl (79) (1.2 g, 4.8 mmol) in acetic anhydride (6 mL) was added pyridine (1 mL, 12.3 mmol). After stirring for 5 h at 25°C, water (20 mL) was added and extracted with CH_2Cl_2 (4 x 20 mL). The CH_2Cl_2 extract was washed with 2N HCl (3 x 8 mL), saturated $NaHCO_3$ solution, water (2 x 8 mL), dried and evaporated to give 1.63 g of (83), as white solid.

yield	:	82%
mp	:	116°C
ir(KBr)ν_{max} cm^{-1}	:	3322, 2956, 1735, 1642, 1530, 1504
nmr($CDCl_3$) δ	:	2.0 (s, 3H, N-$COCH_3$), 2.28 (s, 6H, $OCOCH_3$ × 2), 3.16 (d, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.91 (q, 1H, $C^{\alpha}H$), 6.19 (d, 1H, NH), 6.91-7.22 (m, 3H, NH + aromatic)

LXXXIII. N-Acetyl 3,4-Dihydroxyphenylalanine Methyl Ester (84) :

A solution of (83) (0.66 g, 2 mmol) in MeOH (10 mL) was treated with aqueous $NaHCO_3$ (0.168 g, 2 mmol, 6 mL) and stirred at room temperature for 2 h. The reaction mixture was concentrated to about 6 mL and acidified with 10% HCl, extracted with EtOAc (3 x 15 mL), dried and evaporated to give 0.38 g of (84), as a thick syrup.

yield	:	75%
mp	:	gummy
ir(neat)ν_{max} cm^{-1}	:	3320(br), 1730, 1713, 1695, 1666, 1650, 1537
nmr($CDCl_3$) δ	:	1.9 (s, 3H, $NCOCH_3$), 2.92 (d, 2H, $C^{\beta}H_2$), 3.65 (s, 3H, $COOCH_3$), 4.7 (q, 1H, $C^{\alpha}H$), 5.92 (d, 1H, NH), 6.28-6.85 (m, 3H, aromatic)
anal	:	Calcd. for $C_{12}H_{15}NO_5$ (M.W. 253) C, 56.91; H, 5.91; N, 5.53 % Found : C, 57.87; H, 5.97; N, 5.97 %

LXXXIV. N-Formyl 3,4-Dihydroxy-6-nitro-phenylalanine Methyl Ester (85):

To a stirred and ice-cooled mixture of (80) (0.239 g, 1 mmol) and sodium nitrite (0.152 g, 2.2 mmol) in water (5 mL) was added, in drops, 2.5M H_2SO_4 (0.67 mL, 1.75 mmol). After stirring at room temperature for 2 h the reaction mixture was extracted with EtOAc (5 x 15 mL), washed with water (2 x 5 mL), dried and evaporated. The residue on crystallization from EtOAc-hexane gave 0.235 g of (85), as a yellow solid.

yield	:	83%
mp	:	168-169°C
ir(KBr)ν_{max} cm^{-1}	:	3403, 3322, 1720, 1651, 1597, 1537, 1334, 1308
nmr($CDCl_3$-DMSO-d_6) δ	:	2.94 -3.50 (m, 2H, $C^{\beta}H_2$), 3.72 (s, 3H, $COOCH_3$), 4.88 (m, 1H, $C^{\alpha}H$), 6.81 (s, 1H, ar), 7.28-7.75 (m, 2H, NH + aromatic), 8.13 (s, 1H, CHO)
ms (m/z)	:	285 $(MH)^+$

LXXXV. N-Formyl(O,O'-Diacetyl)-3,4-Dihydroxy-6-nitrophenylalanine Methyl Ester (86) :

To a stirred suspension of (85) (0.1 g, 0.35 mmol) in acetic anhydride (1 mL) was added pyridine (0.1 mL, 1.23 mmol). After stirring for 3 h at room temperature, water (8 mL) was added and extracted with EtOAc (5 x 15 mL). The EtOAc extract was washed with 2N HCl (2 x 8 mL), saturated $NaHCO_3$ solution (4 x 8 mL) and water (2 x 5 mL), dried and evaporated *in vacuo* to give 0.106 g of (86) as reddish yellow gum.

yield	:	82%
mp	:	gummy
ir(neat)ν_{max} cm^{-1}	:	3368, 1734, 1654, 1597, 1534, 1331.
nmr($CDCl_3$) δ	:	2.35 (s, 6H, $OCOCH_3$ × 2), 3.54 (m, 2H, $C^{\beta}H_2$), 3.78 (s, 3H, $COOCH_3$), 5.0 (q, 1H, $C^{\alpha}H$), 6.63 (d, 1H, NH), 7.25 (s,1H, aromatic), 7.89 (s, 1H, aromatic), 8.26 (s, 1H, CHO)

LXXXVI. N-Formyl (4-O-Dimethyl Malonate)-3,4-Dihydroxyphenylalanine Methyl Ester (87) :

To a stirred and ice-cooled solution of N-formyl-DOPA-OMe (80) (0.12 g, 0.5 mmol) in DMF (3 mL) was added NaH (50%, 0.024 g, 1 mmol). After stirring the reddish brown solution for 0.5 h methyl bromo malonate (0.211 g, 1 mmol) in DMF (1 mL) was added to the reaction mixture and stirring continued at room temperature for 8 h. After acidifying the reaction mixture with 2N HCl, water (5 mL) was added, extracted with EtOAc (4 x 10 mL), dried and evaporated *in vacuo*. The crude product on purification through a silica gel column (EtOAc:PhH :: 1:9) gave 0.045 g of (87) as a thick syrup.

yield	:	24%
mp	:	gummy
ir(neat)ν_{max} cm^{-1}	:	3418(br), 2923, 2852, 1733, 1684, 1652, 1558, 1506.
nmr($CDCl_3$) δ	:	3.05 (d, 2H, $C^{\beta}H_2$), 3.74 (s, 3H, dopa ester), 3.85 (s, 6H, malonic $COOCH_3$ × 2), 4.32 (m, 1H, malonic CH), 4.89 (q, 1H, $C^{\alpha}H$), 6.1 (d, 1H, NH), 6.58-6.9 (m, 3H, aromatic), 7.1 (s, 1H, phenolic OH), 8.09 (s, 1H, CHO)
ms (m/z)	:	369 $(M)^+$

LXXXVII. N-Formyl(3-O-Methoxy Ethoxy Methyl)-3,4-Dihydroxyphenylalanine Methyl Ester (88) :

To a stirred solution of N-formyl-DOPA-OMe (80) (0.478 g, 2 mmol) in 10% aqueous Na_2CO_3 (10 mL) was added methoxy ethoxy methyl chloride (MEM chloride) (0.992 g, 8 mmol). After stirring for 3 h at room temperature, the reaction mixture was extracted with EtOAc (5 x 15 mL), washed with water (2 x 5 mL), dried and evaporated *under vacuum*. The residue on purification through a silica gel column (EtOAc:PhH :: 1:4)

gave (88) as a thick syrup.

yield	:	48%
mp	:	gummy
ir(neat)ν_{max} cm^{-1}	:	3340(br), 2950, 1731, 1652, 1594, 1510, 1439
nmr($CDCl_3$) δ	:	3.03 (d, 2H, $C^{\beta}H_2$), 3.44 (s, 3H, OCH_3), 3.53-3.81 (m, 7H, $-CH_2CH_2-$ + $COOCH_3$), 4.69-4.97 (m, 3H, OCH_2O + $C^{\alpha}H$), 6.5 (d, 1H, aromatic), 6.7 (s, 1H, aromatic), 6.81 (d, 1H, aromatic), 7.03 (d, 1H, NH), 8.19 (s, 1H, CHO)
ms (m/z)	:	328 $(MH)^+$

LXXXVIII. Reaction of (80) with Allyl Bromide :

Preparation of N-Formyl(O,O'- Bis Allyl)-3,4-Dihydroxyphenylalanine Methyl Ester (89), N-Formyl(4-O- Allyl)-3,4-Dihydroxyphenylalanine Methyl Ester (90) and N-Formyl (3-O-Allyl) 3,4-Dihydroxyphenylalanine Methyl Ester (91) :

To a stirred mixture of (80) (0.239g, 1 mmol) and K_2CO_3 (0.276 g, 2 mmol) in DMF (5 mL) was added allyl bromide (0.242 g, 2 mmol) at room temperature. After stirring for 6 h, the reaction mixture was filtered, the filtrate was admixed with water (10 mL), extracted with EtOAc (3 x 10 mL), washed with 2N HCl, dried and evaporated. TLC of the residue showed three products (EtOAc, R_f 0.65, 0.55, 0.51). Chromatographic separation on silica gel column (EtOAc:PhH :: 1:4) gave 0.027 g of (89) (R_f 0.65, EtOAc), 0.084 g of (90) (R_f 0.51, EtOAc) and 0.056 g of (91) (R_f 0.55, EtOAc) as thick gummy compounds.

(89)

yield	:	8.5%
mp	:	gummy
ir(neat)ν_{max} cm^{-1}	:	3314(br), 2924, 2856, 1744, 1683, 1513, 1425

nmr($CDCl_3$) δ	:	2.95 (d, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, $COOCH_3$), 4.43 (d, 4H, OCH_2 × 2), 4.79 (q, 1H, $C^{\alpha}H$), 5.0-5.5 (m, 4H, $=CH_2$ × 2), 5.66-6.23 (m, 3H, NH + =CH- × 2), 6.36-6.80 (m, 3H, aromatic), 7.96 (s, 1H, CHO)
ms (m/z)	:	319 $(M)^+$

(90)

yield	:	30%
mp	:	gummy
ir(neat)ν_{max} cm^{-1}	:	3340(br), 2925, 1742, 1672, 1599, 1514, 1437.
nmr($CDCl_3$) δ	:	3.09 (d, 2H, $C^{\beta}H_2$), 3.78 (s, 3H, $COOCH_3$), 4.59 (d, 2H, OCH_2), 4.97 (q, 1H, $C^{\alpha}H$), 5.19-5.63 (m, 2H, $=CH_2$), 5.81-6.44 (m, 3H, NH + =CH- + aromatic C-6 H), 6.53-6.97 (m, 2H, aromatic C-2 H + aromatic C-5 H), 8.22 (s, 1H, CHO)
ms (m/z)	:	280 $(MH)^+$

(91)

yield	:	20%
mp	:	gummy
ir(neat)ν_{max} cm^{-1}	:	3342(br), 3028, 2928, 2953, 1742, 1672, 1595, 1513
nmr($CDCl_3$) δ	:	3.06 (d, 2H, $C^{\beta}H_2$), 3.69 (s, 3H, $COOCH_3$), 4.56 (d, 2H, OCH_2), 4.94 (q, 1H, $C^{\alpha}H$), 5.19-5.59 (m, 2H, $=CH_2$), 5.84-6.31 (m, 2H, NH + =CH-), 6.41-7.0 (m, 3H, aromatic), 8.25 (s, 1H, CHO)
ms (m/z)	:	280 $(MH)^+$

LXXXIX. Monobenzyloxycarbonyl Cystine (92) :

To an ice-cooled and vigorously stirred solution of L-cystine (10 g, 41.7 mmol) in 1.65N NaOH (100 mL) was added benzyloxycarbonyl chloride (95%) (2.63 mL, 19 mmol) over a period of 0.5 h. After an additional 20 min, the reaction mixture was carefully adjusted to pH 6 with 6N HCl and the stirring and cooling continued for 20 min longer. The precipitate of excess of L-cystine was filtered and washed with water (10 mL). The combined filtrate and washings were adjusted to pH 3.2 with 6N HCl and after cooling to 4°C for 10 h was filtered and washed several times with alcohol then ether. Recrystallization could be effected by suspending the material in water (100 mL), bringing the

pH to 6.5 by the addition of 2N NaOH with stirring to effect solution filtering off any undesired material, and then adjusting the pH to 3.2 by the addition of 6N HCl. After cooling, the precipitate was filtered and washed successively with cold water, ethanol and ether. The product on drying weighed 3.24 g.

yield	:	46%
mp	:	196-200°C
ir(KBr)ν_{max} cm^{-1}	:	3360(br), 3060, 1704, 1678, 1523, 1448, 1399
ms (m/z)	:	375 $(MH)^+$

XC. Bis-N-Benzyloxycarbonyl Cystine [Bis-Z-Cystine] (93) :

To an ice-cooled and stirred solution of L-cystine (4.8 g, 20 mmol) in 1N NaOH (100 mL) was added, in drops, benzyloxycarbonyl chloride (95% v/v in toluene) (9 mL, 60 mmol) maintaining the pH throughout at 9-10 by addition of 1N NaOH. The reaction was left stirred for 4 h at 0°C, washed with ether (4 x 25 mL), the pH adjusted to 3 with 6N HCl, filtered and the filtrate extracted with EtOAc (6 x 15 mL). The initially precipitated material was dissolved in warm EtOAc, the combined extracts washed with 0.6N HCl (4 x 25 mL), water (4 x 25 mL), dried and evaporated to give 7.32 g of (93) as white solid.

yield	:	72%
mp	:	118-120°C (lit.[63] mp 114°C)
ir(neat)ν_{max} cm^{-1}	:	3333, 3033, 1694, 1586, 1530, 1455

XCI. Bis-N-^{t}Butyloxycarbonyl cystine [Bis-Boc-Cystine] (94) :

To a stirred solution of L-cystine (2.4 g, 10 mmol) in 1N NaOH (22 mL) was added slowly di-^{t}butyl dicarbonate (4.36 g, 20 mmol) for 2 h, left stirred overnight. The resulting milky solution was extracted with petroleum ether (4 x 15 mL), the organic layer extracted with saturated $NaHCO_3$ (4 x 15 mL), the combined aqueous layers adjusted to pH 1 by careful addition of $KHSO_4$ (8.96 g in 60 mL), extracted with EtOAc (4 x 30 mL), washed with water (3 x 20 mL), dried, evaporated with bath temperature

30°C and dried thoroughly *in vacuo* to afford 2.904 g of bis-Boc-cystine (94).

yield	:	66%
mp	:	137-139°C (lit.[64] mp 143-145°C)
ir(neat)ν_{max} cm^{-1}	:	3368, 2988, 1743, 1718, 1680, 1509, 1410
nmr($CDCl_3$)δ	:	1.47 (s,s, 18H, Boc CH_3 × 2), 3.25 (m, 4H, $C^{\beta}H_2$ × 2), 4.47 (q, 2H,$C^{\alpha}H$ × 2), 5.78 (d, 2H, NH × 2), 6.28 (s, 2H, COOH × 2)

XCII. Monobenzyloxycarbonyl Cystine Bis-Methyl Ester Hydrochloride (95):

A solution of monobenzyloxycarbonyl cystine (1.9 g, 5 mmol) in cold 2N methanolic HCl (15 mL) was held at room temperature for one day and then concentrated to dryness *in vacuo* at 40°C. The evaporation was repeated twice after the addition each time of methanol (25 mL). The residue thereby obtained is dissolved in cold 1N methanolic HCl (15 mL) and the entire procedure repeated. Solution of the final residual material in a little methanol followed by the addition of acetone leads to the precipitation of (95) (1.69 g) as colorless needles.

yield	:	76%
mp	:	149-151 °C (lit.[65] mp 159-160 °C)
ir(KBr)ν_{max} cm^{-1}	:	3375, 2946, 2840, 1732, 1684, 1514
nmr($CDCl_3$-DMSO-d6) δ	:	3.38 (m, 7H, NH_3^+ + $C^{\beta}H_2$ × 2), 3.81 (s, s, 6H, $COOCH_3$ × 2), 4.13 - 4.69 (m, 2H, $C^{\alpha}H$ × 2), 5.13 (s, 2H, Z CH_2), 7.34 (s, 6H, NH + Z aromatic)
ms (m/z)	:	403 $(MH)^+$ -HCl

XCIII. N'-Z-Cystinyl(OMe)$_2$-N"N"-[(bis-Boc)Cystinyl]- Cystine(N'-Z)-diOMe (96) :

1-Hydroxy benzotriazole (HOBt) (0.337 g, 2.5 mmol) followed by a solution of DCC (0.515 g, 2.5 mmol) in CH_2Cl_2 (5 mL) was added to a stirred solution of bis-Boc-cystine (94) (0.55 g 1.25 mmol) in dry CH_2Cl_2 (20 mL). A solution of mono-Z-cystine-diOMe - freshly prepared by dropwise addition of NEt_3 (0.35 mL, 2.5 mmol) to an ice-cooled and stirred solution of mono-Z-cystine-diOMe.HCl (95) (1.095 g, 2.5 mmol)

in dry DMF (15 mL) and leaving aside for 0.5 h - was then added. The mixture was left stirred overnight, filtered, washed with CH_2Cl_2, the filtrate and washings evaporated, the residue dissolved in EtOAc, washed with 2N HCl (2 x 25 mL), saturated NaCl (2 x 15 mL), dried, evaporated and recrystallized from EtOAc-hexane to give 0.94 g of (96) as white powder.

yield	:	62%
mp	:	93°C
ir(KBr)ν_{max} cm^{-1}	:	3341, 2928, 1740, 1692, 1665, 1521, 1456, 1436, 1392.
ms (m/z)	:	1009 $(MH)^+$ -2 Boc
anal	:	Calcd. for $C_{48}H_{68}N_6O_{18}S_6$ (M.W. 1208) C, 47.68; H, 5.62; N, 6.95 % Found : C, 47.84; H, 5.93; N, 7.24 %
uv-vis $(CH_3CN)\lambda_{max}$ nm (ϵ, L mol^{-1} cm^{-1})	:	251 (sh, 1527), 256 (1461), 262 (sh, 1223), 267 (sh, 965), 325 (sh, 78)

^{1}H nmr(400 MHz) studies on (96) :

δ ($CDCl_3$), 24°C	:	1.45 (s, 18H, Boc CH_3 × 6), 3.0 (m, 4H, pep $C^\beta H_2$ × 2), 3.06 - 3.36 (m, 8H, Boc $C^\beta H_2$ × 2 + Z $C^\beta H_2$ × 2), 3.75 (m, 12H, $COOCH_3$ × 4), 4.66 (q, 2H, Z $C^\alpha H$ × 2), 4.79 (q, 2H, Boc $C^\alpha H$ × 2), 4.88 (q, 2H, pep $C^\alpha H$ × 2), 5.12 (s, s, 4H, Z CH_2 × 2), 5.62 (d, 2H, Boc NH × 2), 5.86 (d, 2H, Z NH × 2), 7.36 (s, s, 10H, aromatic), 7.85 (d, 2H, pep NH × 2)
δ(DMSO-d_6),24°C	:	1.38 (s, 18H, Boc CH_3 × 6), 2.5 (DMSO), 2.8, 2.9 (q, q, 4H, pep $C^\beta H_2$ × 2), 3.0 (m, 4H, Boc $C^\beta H_2$ × 2), 3.1 (m, 4H, Z $C^\beta H_2$ × 2), 3.32 (H_2O), 3.65 (s, s, 12H, $COOCH_3$ × 4), 4.21 (m, 2H, Boc $C^\alpha H$ × 2), 4.35 (m, 2H, Z $C^\alpha H$ × 2), 4.55 (m, 2H, pep $C^\alpha H$ × 2), 5.05 (s, 4H, Z CH_2 × 2), 7.05 (d, 2H, Boc NH × 2), 7.35 (s, 10H, aromatic), 7.9 (d, 2, Z NH × 2), 8.45 (d, 2H, pep NH × 2)

XCIV. N'-Z-Cystinyl$(OMe)_2$-N"N"-[(bis-Z)Cystinyl]- Cystine(N'-Z)-diOMe (97) :

1-Hydroxy benzotriazole (HOBt) (0.675 g, 5 mmol) followed by a solution of DCC (1.03 g, 5 mmol) in CH_2Cl_2 (10 mL) was added to a stirred solution of bis-Z-cystine (93)

(1.27 g, 2.5 mmol) in dry CH_2Cl_2 (20 mL). A solution of mono-Z-cystine-diOMe - freshly prepared by dropwise addition of NEt_3 (0.7 mL, 5 mmol) to an ice-cooled and stirred solution of mono-Z-cystine-diOMe.HCl (95) (2.19 g, 5 mmol) in dry DMF (15 mL) and leaving aside for 0.5 h - was then added. The mixture was left stirred overnight, filtered, washed with CH_2Cl_2, the filtrate and washings evaporated, the residue dissolved in EtOAc, washed with 2N HCl (2 x 25 mL), saturated NaCl (2 x 15 mL), dried, evaporated and chromatographed on silica gel. Elution with PhH:EtOAc (3:2) gave 1.65 g of (97) as white powder.

yield	:	52%
mp	:	127-130°C
ir(neat)ν_{max} cm^{-1}	:	3331, 2951, 1736, 1652, 1533
ms (m/z)	:	1277 $(MH)^+$
uv-vis $(CH_3CN)\lambda_{max}$ nm (ϵ, L mol^{-1} cm^{-1})	:	245 (sh, 2218), 251 (2158), 256 (2099), 262 (sh, 1743), 267 (sh, 1347)

^{1}H nmr(400 MHz) studies on (97) :

δ ($CDCl_3$), 24°C	:	2.9 (d, 4H, pep $C^\beta H_2$ × 2), 3.15 (m, 8H, ^{1}N $C^\beta H_2$ × 2 + $^\omega$N $C^\beta H_2$ × 2), 3.70 (s,s, 12H, $COOCH_3$ × 4), 4.62 (q, 2H, ^{1}N $C^\alpha H$ × 2), 4.88 (q, 2H, pep $C^\alpha H$ × 2), 5.1 (s, s, 8H, Z CH_2 × 4), 5.15 (dd, 2H, $^\omega$N $C^\alpha H$ × 2), 5.8 (d, 2H, ^{1}NH × 2), 5.9 (d, 2H, $^\omega$ NH× 2), 7.3 (m, 20H, aromatic), 8.15 (d, 2H, pep NH × 2)
δ(DMSO-d_6),24°C	:	2.85 (d d, 4H, pep $C^\beta H_2$ × 2), 2.95 (brm, 4H, $^\omega$N $C^\beta H_2$ × 2), 3.1 (m, 4H, ^{1}N $C^\beta H_2$ × 2), 4.32 (H_2O), 3.65 (s, s, 12H, $COOCH_3$ × 4), 4.35 (m, 4H, ^{1}N C^α × 2 + $^\omega$N $C^\alpha H$ × 2), 4.55 (q, 2H, pep $C^\alpha H$ × 2), 5.05 (s, 8H, Z CH_2 × 4), 7.3 (s, 20H, aromatic), 7.6 (d, 2H, $^\omega$NH × 2), 7.9 (d, 2H, ^{1}NH × 2), 8.6 (d, 2H, pep NH × 2)

XCV. Bis-Salicylaldehyde - Cystine-diOMe Schiff Base (98) :

To a stirred methanolic solution (25 mL) of cystine-diOMe.2HCl (1.705 g, 5 mmol) was added, in drops, NEt_3 (1.39 mL, 10 mmol). After 10 min salicylaldehyde (1.05 mL, 10 mmol) was added, the reaction mixture held at 50°C for 0.5 h and solvents evaporated

under vacuo. The residue was chromatographed on a silica gel column. Elution with hexane-PhH (1:1) afforded 1.11 g of the Schiff base (98), (R_f 0.55, EtOAc:PhH :: 1:4) as a reddish gum.

yield	:	47%
mp	:	gummy
ir(neat)ν_{max} cm^{-1}	:	2954, 1742, 1664, 1624, 1594, 1490, 1458
nmr($CDCl_3$)δ	:	1.47 (d, 4H, cystine $C^{\beta}H_2$), 3.31 - 4.19 (m, 8H, $COOCH_3$ + cystine $C^{\alpha}H$), 6.66 - 7.41 (m, 8H, aromatic), 8.31 (s, 2H, -N=CH), 12.16 (s, 2H, OH)

XCVI. Zinc Complex of Bis-Salicylaldehyde - Cystine-diOMe Schiff Base (99):

To a stirred methanolic solution (15 mL) of Schiff base (98) (0.1 g, 0.21 mmol) under nitrogen atmosphere was added PDT (0.033 g, 0.315 mmol). After stirring for 8 h at rt, solvents were evaporated, residue washed with hexane (4 × 10 mL), redissolved in MeOH (10 mL) and NEt_3 (0.042 g, 0.42 mmol), $ZnCl_2$ (0.057 g, 0.42 mmol) were added successively. The zinc complex (99) precipitated as a white solid, which was filtered and dried (0.068 g).

yield	:	54%
mp	:	> 340°C
ir(KBr)ν_{max} cm^{-1}	:	3420, 3058, 2925, 1724, 1621, 1548, 1477, 1446
ms (m/z)	:	605 $(MH)^+$

F. REFERENCES

1. Tous, G.; Bush, A.; Tous, A.; Jordan, F. *J. Med. Chem.* **1990**, *33*, 1620.

2. Gergely, A. and Kiss, T., In " Metal Ions in Biological Systems", Vol.9; Sigel, H., Ed.; Marcel Dekker: New York, 1979; Chap. 5.

3. Vallee, B.L. and Wacker, W.E.C., In "The Proteins", Vol.5; 'Metalloproteins' Neurath, H., Ed.; Academic Press: New York and London, 1970, pp 54.

4. Musacchio, J.M. In "Handbook of Psychopharmacology", Vol.3; 'Biochemistry of Biogenic Amines' Iversen, L.L., Iversen, S.D. and Synder, S.H., Eds.; Plenum Press: New York and London, 1975, pp 1.

5. Waser, E.; Lewandowski, M. *Helv. Chim. Acta,* **1921**, *4*, 657.

6. Bretschneider, H.; Hohenlohe-Oehringen, K.; Kaiser, A.; Wolcke, U. *Helv. Chim. Acta,* **1973**, *56*, 2857.

7. Nakano, H.; Suzuki, T. *Chem. Abstr.* **1978**, *88*, P 62608e.

8. Konda, M.; Shiroiri, T.; Yamada, S. *Chem. Pharm. Bull.* **1975**, *23*, 1063.

9. Boger, D.L.; Yohannes, D. *J. Org. Chem.* **1987**, *52*, 5283.

10. Riordan, J.F.; Sokolovsky, M.; Vallee, B.L. *J. Am. Chem. Soc.* **1966**, *88*, 8104.

11. Hope, D.B.; Walti, M. *Biochem. J.* **1973**, *135*, 241.

12. Stewart, F.H.C. *Aust. J. Chem.* **1979**, *32*, 661.

13. Larsen, P.O.; Kjar, A. *Acta Chem. Scand.* **1962**, *16*, 142.

14. Arnold, Z.; Larsen, P.O. *Acta Chem. Scand. B* **1977**, *31*, 826.

15. Ito, S.; Inoue, S.; Yamamoto, Y.; Fujita, K. *J. Med. Chem.* **1981**, *24*, 673.

16. Solar, S.L.; Schumaker, R.R. *J. Org. Chem.* **1966**, *31*, 1996.

17. Alewood, P.F.; Johns, R.B.; Velerio, R.M. *Synthesis* **1983**, 30.

18. Sipos, P.; Kiss, T. *J. Chem. Soc., Dalton Trans.* **1990**, 2909.

19. Mason, H.S. In "The biochemistry of copper", Peisach, J., Aisen, P. and Blumberg, W.F. Eds.; Academic Press: New York, 1966, pp 340.

20. Rao, T.R.; Sahay, M.; Aggarwal, R.C. *Indian J. Chem.* **1984**, *234*, 214.

21. Haurowitz, F. In "Biochemistry", John Wiley and sons, Inc.: New York, N.Y., 1955, pp 161, 392.

22. Lerner, A.B. *Advances in Enzymol.* **1953**, *14*, 116.

23. Barbeau, A. *Annu. Rev. Pharmacol.* **1974**, *14*, 91.

24. Bodor, N.; Sloan, K.B.; Higuchi, T.; Sasahara, K. *J. Med Chem.* **1977**, *20*, 1435.

25. Abrams, W.B.; Coutinho, C.B.; Leon, A.S.; Spiegel, H.E. *J. Am. Med. Assoc.* **1971**, *218*, 1912.

26. Hinterberger, H. *Biochem. Med.* **1971**, *5*, 412.

27. Banerjee, S.N.; Ressler, C. *J. Org. Chem.* **1976**, *41*, 3056.

28. Ihara, M.; Tsuchiya, Y.; Sawasaki, Y.; Hisaka, A.; Takehana, H.; Tomimoto, K.; Yano, M. *J. Pharm. Sci.* **1989**, *78(7)*, 529.

29. Ihara, M.; Nakajma, S.; Hisaka, A.; Tsuchiya, Y.; Sukuma, Y.; Suzuki, H.; Kitani, K.; Yano, M. *J. Pharm. Sci.* **1990**, *79(8)*, 703.

30. Berthet, M.; Sonveaux, E. *J. Chem. Soc., Chem. Commun.* **1983**, 10.

31. Berthet, M.; Sonveaux, E. *Biopolymers* **1986**, *25*, 189.

32. Kolsa, T.; Miller, M.J. *J. Org. Chem.* **1990**, *55*, 4246.

33. Felix, A.M.; Winter, D.P.; Wang, S-S.; Kulesha, I.D.; Pool, W.R.; Hane, D.L.; Sheppard, H. *J. Med. Chem.* **1974**, *17*, 422.

34. Fuller, W.D.; Verlander, M.S.; Goodman, M. *Biopolymers* **1978**, *17*, 2939.

35. Yamamoto, H.; Hayakawa, T. *Macromolecules* **1976**, *9*, 532.

36. Ranganathan, S.; Tamilarasu, N. *Tetrahedron Lett.* **1994**, *35*, 447.

37. Solomon, E.I.; Baldwin, M.J.; Lowery, M.D. *Chem. Rev.* **1992**, *92*, 521.

38. Gauss, J.M.; Freeman, H.C. *J. Mol. Biol.* **1983**, *169*, 521.

39. Baker, E.N. *J. Mol. Biol.* **1988**, *203*, 1071.

40. Ito, N.; Phillips, S.E.V.; Stevens, C.; Ogel, Z.B.; McPherson, M.J.; Keen, J.N.; Yadav, K.D.S.; Knowles, P.F. *Nature* **1991**, *350*, 87.

41. Tainer, J.A.; Getzoff, E.D.; Richardson, J.S.; Richardson, D.C. *Nature* **1983**, *306*, 284.

42. Kaiser, E.T. *Angew. Chem. Int. Ed. Eng.* **1988**, *27*, 913.

43. Bretschneider, H.; Biemann, K. *Monatsh* **1950**, *81*, 647.

44. Costes, J.P.; Cros, G.; Drabieu, M.H.; Laurent, J.P. *Inorg. Chem. Acta.* **1982**, *60*, 111.

45. Pettit, G.R.; Gupta, S.K.; Ode, R.H. *J. Chem. Soc., Perkin Trans. 1* **1973**, 950.

46. Harris, J.I.; Fruton, J.S. *J. Biol. Chem.* **1951**, *191*, 143.

47. Scoffone, E.; Rocchi, R.; Vidali, G.; Scatturin, A.; Marchiori, F. *Gazz. Chim. Ital.* **1964**, *94*, 743.

48. De Tar, D.F.; Rogers, Jr. F.F.; Bach, H. *J. Am. Chem. Soc.* **1967**, *89*, 3039.

49. Vanngard T., In " Biological Applications of Electron Spin Resonance", Swartz, H.W., Bolton, J.R. and Borg, D.C., Eds.; Wiley: New York, 1972; Chap. 9.

50. Rist, G.H.; Hyde, J.S.; Vanngard, T. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 79.

51. Malmstrom, B.G.; Reinhammar, B.; Vanngard, T. *Biochim. Biophys. Acta.* **1970**, *205*, 48.

52. Stigbrand, T.; Malmstrom, B.G.; Vanngard, T. *FEBS Lett.* **1971**, *12*, 260.

53. Hare, J.W.; Solomon, E.I.; Gray, H.B. *J. Am. Chem. Soc.* **1976**, *98*, 3205.

54. Blumerg, W.E.; Peisach, J. *Biochim. Biophys. Act* **1966**, *126*, 269.

55. Bruner, J. *Chem. Soc. Rev.* **1993**, 183.

56. Johnson, T.B.; Kohmann, E.F. *J. Am. Chem. Soc.* **1915**, *37*, 1863.

57. Hanson, R.W.; Law, H.D. *J. Chem. Soc.* **1965**, 7297.

58. Volger, K.; Baumgartner, H. *Helv. Chim. Acta* 1952, *223*, 1776.

59. Maruyama, K.; Tanimoto, I.; Goto, R. *J. Org. Chem.* **1967**, *32*, 2516.

60. Ranganathan, S.; Jayaraman, N. *Tetrahedron* **1992**, *48*, 931; Ranganathan, S.; Jayaraman, N. *Tetrahedron Lett.* **1992**, *33*, 6681; Ranganathan, S.; Jayaraman, N.; Roy, R.; Madhusudanan, K.P. *Tetrahedron Lett.* **1993**, *34*, 7801.

61. Watson, A.D.; Rao, C.P.; Dorfman, J.R.; Holm, R.H. *Inorg. Chem.* **1985**, *24*, 2820.

62. Marshall, R.; Winitz, M.; Birnbaum, S.M.; Greenstein, J.P. *J. Am. Chem. Soc.* 1957, *79*, 4538.

63. Du Vigneaud, V.; Miller, G.L. *Biochem. Prep.* **1952**, *2*, 74.

64. Keller, O.; Keller, W.E.; Van lokk, G.; Wersin, G. *Org. Syn.* , *63*, 167.

65. Zervas, L.; Bensiton, L.; Weiss, E.; Winitz, M.; Greenstein, J.P. *J. Am. Chem. Soc.* **1954**, *81*, 1729.